JPP Journal of Pharmacy and Pharmacology

JPP 2003, 55; 1213–1221

Received February 12, 2003
Accepted May 20, 2003
DOI 10.1211/0022357021611
ISSN 0022-3573

Investigation of the physical properties of spray-dried stabilised lysozyme particles

Yong-Hong Liao, Marc B. Brown, Abdul Quader and Gary P. Martin

Abstract

The aim of this study was to investigate the effect of the composition of formulations on the physical properties, including glass-transition temperatures (Tg) and aerodynamic-related characteristics, of spray-dried lysozyme particles. The Tg, as determined by differential scanning calorimetry, of spray-dried lysozyme formulations was found to be dependent upon the type and amount of excipient(s) included in the formulation. In addition, the Tg of sucrose-containing particles appeared to be raised markedly by the inclusion of trehalose, but not by dextran. The surfaces of all spray-dried particles were shown by scanning electron microscopy to be smooth with some containing characteristic dimples, typical of spray-dried material, and the morphology appeared to be independent of variation in excipient composition. However, the volume median diameters (VMD) of spray-dried powders, as determined by laser diffraction, were found to depend upon the amounts of excipients. The fine particle fraction of enzyme delivered to the lower stage of a twin-stage impinger from lysozyme–trehalose 1:1 powders appeared to be greater than that from lysozyme–sucrose 1:1 particles (22.5% vs 15.9%) when dispersed via a Rotahaler although a similar dispersibility of the two formulations (39.6% vs 36.7%) was found from a glass inhaler. In general, spray-drying was demonstrated to be feasible to produce respirable particles of the stabilised model protein, with Tg of the formulations being >30°C higher than room temperature.

Introduction

Protein and peptide drugs are generally delivered parenterally. However, the clinical use of these drugs sometimes requires repetitive injections, which potentially makes parenteral dosing undesirable. As a result, efforts to deliver proteins and peptides by non-parenteral routes have been focused on oral, nasal, transdermal, buccal, rectal, vaginal and pulmonary delivery (Wearley 1991; Cleland et al 2001). Of these routes, pulmonary delivery seems to be most promising due to the large surface area of the lung and the absorption efficiency at the site. However, for delivery to the deep lung, an aerodynamic particle size of 1–3 μm is considered desirable (Adjei & Garren 1990). In other words, if respirable powders are to be produced, desirable aerodynamic properties have to be generated and these, in turn, are determined both by the powdering process (e.g. micronisation, spray-drying, etc.) itself and the composition of formulations. In addition, proteins and peptides, unlike traditional small drugs, have secondary, tertiary and quaternary structures, which cause potential lability during processing. Therefore, to develop a formulation containing such biopharmaceuticals it is essential to ensure that resultant preparation is stable during shipping, long-term storage and delivery. In general, disaccharides, such as sucrose and trehalose, are the most extensively utilised and are regarded as the most effective excipients in preserving protein native structure during spray-drying (Labrude et al 1989; Broadhead et al 1994; Adler & Lee 1999; Branchu et al 1999; Murray & Liang 1999; Tzannis & Prestrelski 1999; Lopez-Diez & Bone 2000; Liao et al 2002).

Apart from the preservation of the native structure during spray-drying, the long-term stability of a protein formulation requires the glass transition temperature (Tg) to be well above the storage temperature (Chang et al 1996; Crowe et al 1998; Allison et al 2000). However, the spray-drying process usually leads to the production of protein

MedPharm, Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK

Yong-Hong Liao

Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK

Marc B. Brown, Abdul Quader, Gary P. Martin

Correspondence: G. P. Martin, Department of Pharmacy, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 9NN, UK. E-mail: gary.martin@kcl.ac.uk

Funding: Y.-H. Liao is grateful to Universities UK and MedPharm Ltd, and A. Quader to the Engineering and Physical Sciences Research Council (EPSRC) for their financial support.

powders containing a higher moisture content than those prepared by freeze-drying. For example, when either a humanised monoclonal antibody (anti-IgE) or recombinant humanised deoxyribonuclease were spray-dried so as to achieve a moisture content of $< 3\%$ (which is comparable with freeze-dried formulations), either a high inlet temperature or an additional vacuum drying process was required (Maa et al 1998a). In another study, the moisture content of spray-dried β-galactosidase was found to be 5% when the outlet temperature was 95 °C (Broadhead et al 1994). Such a relatively high moisture content could destabilise both the physical and chemical stability of the protein formulation (Chan & Gonda 1998; Maa et al 1998a). For example, the adsorption of moisture to a glassy formulation can result in a decrease in the Tg of the formulation, and even the crystallisation of the stabilising excipient (Chan & Gonda 1998), which in turn promotes chemical instability of the protein (Maa et al 1998a).

For spray-dried powders intended to be delivered by inhalation, the final aerodynamic properties of the product are as important as the requirement for the protein to be stable. Studies have demonstrated that spray-dried particles are suitable for pulmonary delivery because the size, density and shape of such particles can be controlled by the spray-drying process (Broadhead et al 1994; Chan et al 1997; Maa et al 1998b). For example, the geometric mean particle size can be controlled so that it is between 2 and 5 μm. The aerodynamic performance of spray-dried particles has been evaluated using impinger or impactor systems after dispersion using dry powder inhaler devices (Bosquillon et al 2001; Maa et al 1998a). The fine particle fraction (FPF) of spray-dried powders has been reported to vary by 5–50% or more, and be dependent upon many variables including formulation composition, particle size, shape, density, particle-to-carrier mixing ratio and the inhaler device utilised.

Andya et al (1999) investigated the effects of trehalose, lactose and mannitol on the aerosol performance of spray-dried powders of recombinant humanised monoclonal antibody anti-IgE (rhuMAbE25). The formulation with trehalose was found to be cohesive in nature and this resulted in a poor aerosol performance. The addition of lactose during spray-drying conferred a suitable aerosol performance, although it led to the glycosylation of protein during long-term storage. Mannitol at a specific molar ratio (mannitol–protein, 200:1) in the formulation exhibited an acceptable aerosol performance. In another study, the aerosol performance of rhuMAbE25 spray-dried with mannitol was also found to be mannitol concentration dependent. Upon storage, the fine particle fractions obtained from the powders containing 30% w/w mannitol drastically decreased as a function of time due to crystallisation of the excipient, whereas those containing 10 or 20% mannitol were found to be stable (Costantino et al 1998).

Protein formulations intended for pulmonary delivery require that the native structure of the proteins be protected, the physical properties maximised and the aerodynamic characteristics optimised. However, studies in the literature have often only studied one or two of those factors. Thus, the main objective of this study was to investigate effects of the formulation compositions on the physical (Tg) and aerodynamic-related (particle size, morphology and aerosol performance) properties of spray-dried formulations, with a view also to comparing the effects of incorporating two of the most commonly employed protein-stabilising excipients, sucrose and trehalose. Lysozyme was employed as a model protein since the effect of excipients in preserving the native structure in the solid form and recovered biological activity when processed by spray-drying have been reported elsewhere (Liao et al 2002).

Materials and Methods

Materials

The buffer phosphate salts, sucrose, trehalose, dextran (MW $\sim$ 70 000), lecithin, phosphorus pentoxide, lithium chloride, magnesium chloride, potassium carbonate, potassium bromide, sodium chloride, sodium phosphate and lysozyme ($3\times$ crystallised, dialysed and lyophilised) were purchased from Sigma-Aldrich Co. (UK). Cyclohexane was obtained from BDH (UK). All chemicals were of analytical grade, except lyszoyme.

Spray-drying procedure

The spray-drying procedure employed was that described previously by Liao et al (2002). Briefly, lysozyme and excipients were dissolved in buffer and spray-dried using a Model 190 Buchi mini spray-dryer. The processing parameters comprised a feed rate of 3 mL min^{-1}, an atomising air-flow rate of 700 L h^{-1} and an inlet temperature of 120 °C. Outlet temperatures were found to range from 85 to 90 °C.

Particle size analysis by a laser diffraction analyser

Several milligrams of spray-dried powders were dispersed in 1 mL of 0.1% w/v lecithin cyclohexane solution and the suspension sonicated for 30 s employing a water bath (Model F5100b; Decon Laboratories, Hove, UK) so as to disperse any possible agglomerates before adding a sample to a stirred sample cell. The particle size of the sample was measured by a Malvern 2600 laser diffraction analyser (Malvern Instruments, UK) using a 63-mm focal-length lens at an obscuration of 0.165–0.25 and fitting the data to an independent model. Particle size distributions were expressed in terms of volume median diameter (VMD) and span. The VMD was the diameter at the 50% point of the entire volume distribution while the span was defined as [D(v,90) − D(v,10)]/ D(v,50), where D(v,90), D(v,50) and D(v,10) are the respective diameters at 90, 50 and 10% cumulative volumes.

Scanning electron microscopy (SEM)

Particle size and morphology were investigated using SEM. Double-sized adhesive tape was placed on an

aluminium stub and after stripping off the upper side of the adhesive protection, a small amount of particles were scattered on the stub and dispersed by tapping lightly on the edge of the stub with a spatula to break agglomerates. The particles were then coated with approximately 15–20 nm gold using a sputter coater (Polaron E5100; Polaron Equipment Ltd, Watford, UK) operated at an electrical potential of 15.0 kV, 20 mA. Several photomicrographs were produced by scanning fields, selected randomly, at several magnifications with a Philips SEM501B scanning electron microscope (Einhoven, Netherlands).

Equilibrium moisture sorption

Approximately 100 mg of the powdered samples (×4) were placed into pre-weighed 7 mL glass vials and accurately weighed. The vials then were placed into a desiccator at room temperature containing phosphorus pentoxide, to achieve a relative humidity (r.h.) of 0%. The weight gain or loss of the sample was measured gravimetrically until the change in weight was less than about 0.2% (~0.2 mg) in 24 h. This procedure was then repeated a number of times using the same vials of the same sample formulation but after transferring the vials to desiccators at room temperature containing saturated lithium chloride, magnesium chloride, potassium carbonate, potassium bromide, sodium chloride or sodium phosphate solution. The r.h. of the environment in each desiccator was determined using a hygrometer to be 11, 33, 43, 58, 73 and 97%, respectively. The ambient temperature was found to be 20–26 °C. The moisture content of the samples after equilibration was calculated as grams of water per gram of sample (g g^{-1}).

Deposition test

Spray-dried powders were blended with coarse lactose (45–90 μm) in different mass ratios by hand-mixing. An in-vitro determination of the FPF of lysozyme from aerosolised formulations was carried out using a twin-stage impinger (TSI, Apparatus A; British Pharmacopoeia, 2000). A commercially available simple inhaler device, the Rotahaler, and a glass inhaler developed for experimental purposes (MacRitchie 1998) were used in this experiment. Each deposition via a Rotahaler involved the aerosolisation of the content of 3 capsules, each of the capsules containing a nominal dose of 25 mg powder equivalent to 1.6 mg lysozyme, while two doses (2 × 25 mg) of the blend powders were individually dispersed from the glass inhaler for each deposition experiment. The powder was aerosolised at 60 L min^{-1} during the 7-s operation of the vacuum pump. After aerosolisation of the formulation, the inhaler device, the mouthpiece-adapter and capsule shells (if present) were washed with deionised water. The amount of lysozyme remaining in the device, and depositing on stages 1 or 2 was determined using the HPLC assay described previously (Liao et al 2001). The amount depositing on each stage was expressed as a percentage of the recovered lysozyme out of the nominal amount of the total enzyme in the formulation.

Statistical analysis

All data are expressed as means ± standard deviations (s.d.). Statistical analysis of the effects of sugar-to-lysozyme mass ratio on the Tg and particles size of the sugar-containing formulations was performed using the Kruskal Wallis test (Minitab; Minitab Inc., CA), and of the effects of the device and the formulations on the fine particle fraction of the tested formulations using a two-way analysis of variance (Minitab; Minitab Inc., CA). In all cases, P values less than 0.05 were considered statistically significant.

Results

Effects of excipient(s) on the Tg and sucrose crystallisation temperature (Tc) of the resultant spray-dried formulations

Differential scanning calorimetry (DSC) thermograms (data not shown) of all spray-dried formulations contained an endotherm between 120 and 150 °C corresponding to the lysozyme melting or denaturation peak. For formulations with a sugar-to-enzyme mass ratio more than 1:5, a glass transition was also observed. When sucrose was included as an excipient, an exothermal peak attributable to sucrose crystallisation was sometimes apparent in the DSC thermograms, whereas no crystallisation events were detected in non-sucrose-containing formulations.

For both sucrose- and trehalose-containing formulations, the Tg values generally appeared to increase with increasing sugar content (Table 1). Trehalose conferred a higher Tg than sucrose did on a weight-by-weight basis. The Tgs of the former samples were 10–20 °C higher than the equivalent values obtained when sucrose was employed as an excipient. The effect of sugar-to-lysozyme mass ratio on the Tg of the formulation was found to be significant for those containing sucrose ($P < 0.05$) but not for those containing trehalose ($P > 0.05$).

The Tc of sucrose also appeared to show some correlation with the relative amount included in the formulations inasmuch as only two formulations exhibited a crystallisation peak in the DSC thermograms. The Tcs of the two formulations, namely with sucrose-to-lysozyme mass ratios of 4:1 and 10:1, were determined to be 149.6 ± 4.7 and 131.7 ± 3.4 °C (n = 3), respectively, while the crystallisation enthalpies (ΔHcs) were 42.6 ± 10.1 and 89.5 ± 5.4 J g^{-1} (n = 3), respectively. In contrast, there was no crystallisation peak found in the thermograms obtained for any of the trehalose containing formulations.

The effects on Tg of part or full replacement of sucrose in sucrose-containing lysozyme formulations, with either trehalose or dextran, were also investigated and the data are shown in Table 2. In all these formulations, there appeared to be no sucrose crystallisation on the basis of the DSC thermograms obtained. The Tgs of formulations

Table 1 The glass transition temperature (Tg) of lysozyme–sucrose/trehalose powders spray-dried from solutions containing 5 mg mL^{-1} of enzyme.

Sugar-to-lysozyme mass ratio	Tg of sucrose-containing formulation (°C)	Tg of trehalose-containing formulation (°C)
0:1	ND	ND
1:10	ND	ND
1:5	ND	ND
2:5	34.2 ± 4.1	46.0 ± 0.9
1:1	51.4 ± 1.5	68.6 ± 1.9
2:1	48.6 ± 2.6	67.6 ± 5.6
4:1	52.1 ± 3.6	66.7 ± 5.3
10:1	58.0 ± 1.2	72.0 ± 3.9

Data are means ± s.d., n = 3. ND, not detected.

Table 2 The glass transition temperature (Tg) of lysozyme–sucrose–trehalose and lysozyme–sucrose–dextran powders spray-dried from solution containing 5 mg mL^{-1} of enzyme.

Lysozyme-to-sucrose-to-trehalose mass ratio	Tg (°C)[a]	Lysozyme-to-sucrose-to-dextran mass ratio	Tg (°C)[b]
5:10:0	44.9 ± 1.2	5:5:0	60.6 ± 0.8
5:8:2	48.5 ± 4.3	5:4:1	57.6 ± 2.2
5:6:4	50.0 ± 2.8	5:3.5:1.5	56.9 ± 2.5
5:4:6	52.6 ± 0.4	5:2.5:2.5	59.6 ± 1.3
5:2:8	59.8 ± 0.3	5:1.5:3.5	60.3 ± 1.1
5:0:10	63.6 ± 1.6	5:0:5	ND

Data are means ± s.d., n = 3. ND, not detected. [a]The particles were produced from a solution spray-dried at an inlet temperature of 95 °C as opposed to 120 °C for other samples with moisture contents ranging from 3.3% to 3.9% w/w. [b]The moisture content of lysozyme–sucrose–dextran formulations was equilibrated to 1.5–4.0% w/w as a consequence of storage in a phosphorus-pentoxide-containing desiccator (0% r.h.).

containing mixtures of sucrose and trehalose increased significantly with increasing trehalose content ($P < 0.05$) when the total concentration of the excipient(s) was held constant at 10 mg mL^{-1} (Table 2). The Tg of the formulation containing 10 mg mL^{-1} of trehalose was found to be 19 °C higher than that of the equivalent lysozyme–sucrose formulation. In addition, when the content of sucrose was replaced by 40–60% trehalose, the Tg of the formulation increased by 5–8 °C. The variation in the moisture content of the different lysozyme–sucrose–dextran batches were minimised by storage in a phosphorus-pentoxide-containing desiccator. The stepped replacement of sucrose with dextran was not found to alter the Tg significantly ($P < 0.05$), although the Tg of formulations of lysozyme with dextran alone could not be determined (Table 2).

The moisture sorption of lysozyme–sugar formulations as a function of relative humidity (r.h.) is shown in Figure 1. Three different formulations, namely spray-dried lysozyme–sucrose 1:1, lysozyme–trehalose 1:1 and lysozyme–trehalose–sucrose 5:2.5:2.5, produced similar water sorption isotherms. In the range of 0–58% r.h., the moisture uptake for all three formulations was found to be almost identical. However, at 73% r.h., the moisture content for the formulation containing lysozyme–sucrose 1:1 was found to be 25.0% w/w, which was slightly higher than those of the other two formulations. When the three formulations were stored at 97% r.h., all three formulations virtually dissolved. The moisture content of lysozyme–sucrose 1:1, lysozyme–trehalose 1:1 and lysozyme–sucrose–trehalose 5:2.5:2.5 at this high level of humidity was found to be 65.3, 53.5 and 66.1% w/w, respectively. The study of moisture desorption was not completed due to the crystallisation of sugars when stored at 73% r.h. after being pre-equilibrated at 97% r.h.

Moisture uptake was found to affect the Tg of the three formulations. In agreement with previous reports (Slade & Levine 1988; Roos & Karel 1991; Saleki-Gerhardt & Zografi 1994), the Tg of the formulations decreased drastically with increasing water uptake (data not shown). When the moisture content of the formulations increased from ~1.5%, as occurred when samples were equilibrated at 0% to ~4% r.h., as obtained after storage at 11% r.h., the Tgs of lysozyme–sucrose 1:1 and lysozyme–trehalose 1:1 formulations were found to decrease by more than 20 °C. After equilibration of these latter two formulations at 43% r.h., the Tg decreased to approximately 20 °C or below.

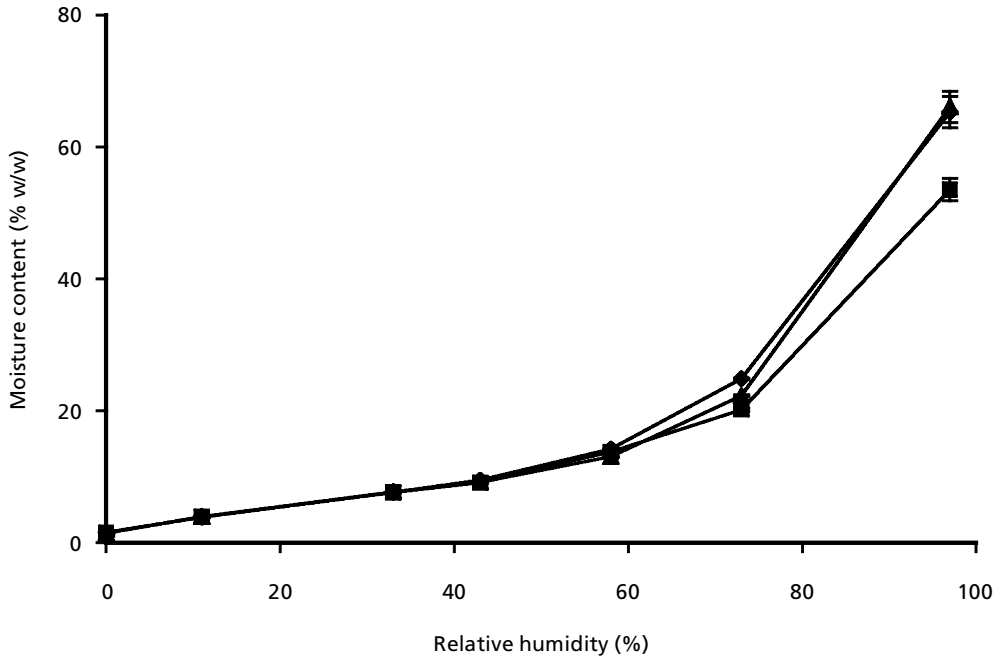

Figure 1 Moisture uptake isotherms of different spray-dried formulations as a function of relative humidity: ◆, lysozyme–sucrose 1:1; ■, lysozyme–trehalose 1:1; ▲, lysozyme–sucrose–trehalose 5:2.5:2.5 (mean ± s.d., n = 4).

Effects of excipient(s) on the aerodynamic properties of spray-dried formulations

The morphology of spray-dried particles appeared to generally be similar and independent of excipient type and concentration as shown in scanning electron microscopy (SEM) results (Figure 2). Visually, the surface of all particles was smooth, although some contained typical dimples of spray-dried materials.

The volume median diameters (VMD) of spray-dried powders determined by laser diffraction were found to be significantly affected by the amount of excipient ($P < 0.05$) (Figure 3). The addition of sucrose or trehalose up to 5–10 mg mL^{-1} to the solution of lysozyme before spray-drying (which corresponded to a lysozyme-to-sugar mass ratio of 1:1 to 1:2) appeared to decrease the resultant particle size. However, a further increase in the concentration of excipient led to an increase in the particle size. The span of particle size distribution was found to be independent of excipient content and varied between 0.86 and 1.41, which indicated that all powders exhibited a high degree of monodispersity. In addition, the particle size was not significantly affected ($P > 0.05$) by the stepwise replacement of sucrose with either dextran or trehalose when the mixture of sucrose and dextran/trehalose was maintained constant. For example, the VMD and span of spray-dried sucrose–dextran–lysozyme particles were found to vary between 3.35 and 3.61 μm and between 0.87 and 1.02, respectively (Table 3).

In pilot deposition studies, a series of formulations were produced containing drug (spray-dried lysozyme–sucrose 1:1 particles)-to-carrier mass ratios of 1:0, 1:3, 1:6, 1:15 and 1:67.5, and evaluated as dispersed via a glass inhaler. The corresponding FPFs were determined to be about 13, 27, 43, 42 and 40%, respectively. On the basis of these results, where the FPF appeared to be maximised by drug-to-carrier ratio of 1:6, this latter ratio was employed in subsequent deposition studies to determine the effects of sugar excipient and device on FPF (Table 4). The FPF of the studied formulations appeared to be significantly affected by the devices utilised, while the difference between sucrose- and trehalose-containing formulations was found to be insignificant (two-way analysis of variance). When dispersed via a Rotahaler, the FPF, which was 22.5% and 15.9% for lysozyme–trehalose 1:1 powder and lysozyme–sucrose 1:1 particles, respectively, was found to be lower than that achievable when the same two formulations were aerosolised from a glass device (Table 4).

Discussion

The effects of excipients on the Tg of lysozyme-containing powders appeared to be more complicated when produced by spray-drying, as in this study, compared with those on the Tg of freeze-dried formulations. In the freeze-dried formulations, the stepped replacement of sucrose with either trehalose (unpublished data) or dextran (Allison et al 2000) led to an increase in the Tg of sucrose-stabilised lysozyme, while increasing sucrose content per se in a lysozyme powder resulted in a lowered Tg. However, unlike the freeze-dried powders, the Tg of spray-dried sucrose–lysozyme formulations appeared to be almost the same as the sucrose was replaced with stepwise

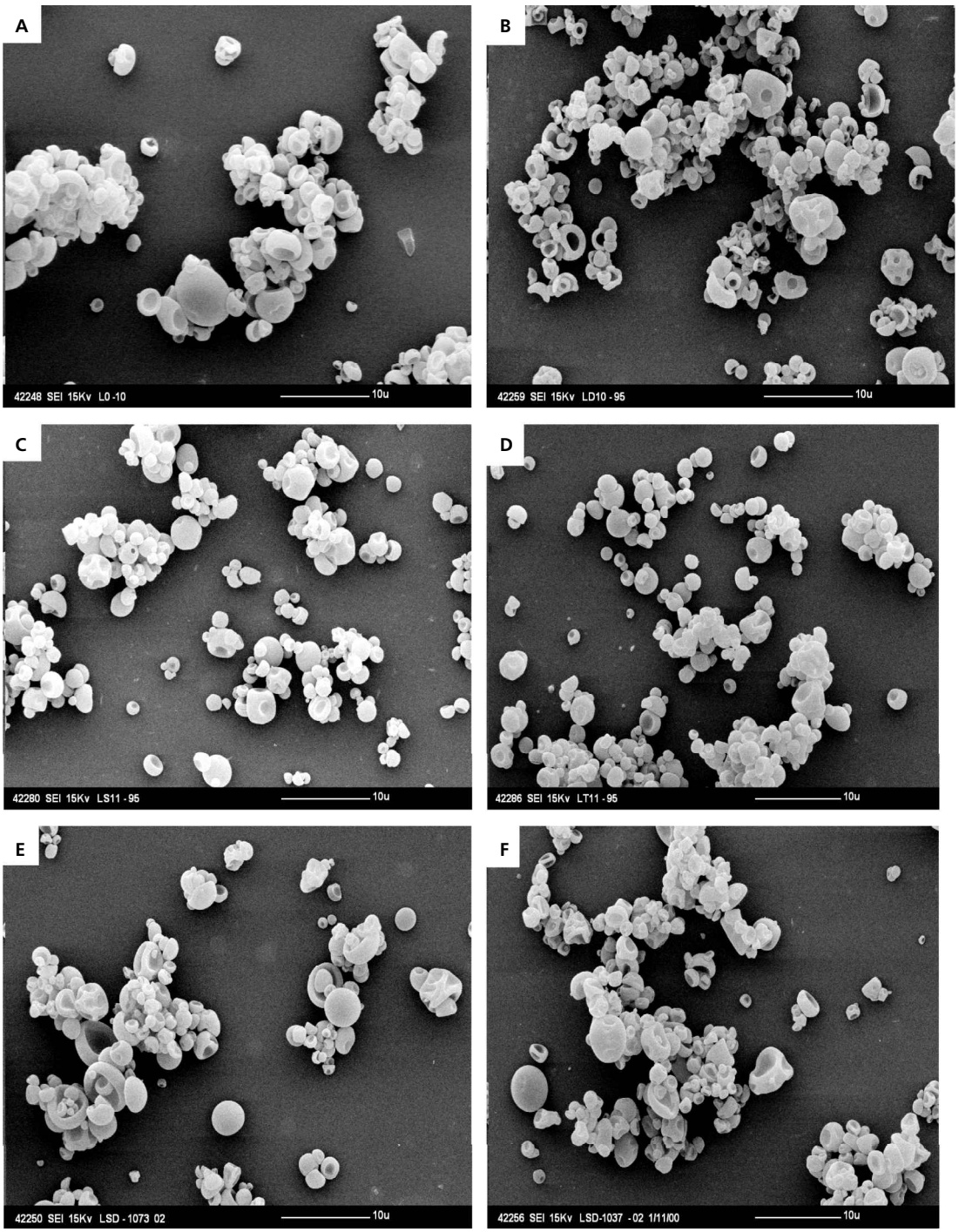

Figure 2 Scanning electron micrographs of spray-dried particles. A. lysozyme alone. B. lysozyme–dextran 1:1. C. lysozyme–sucrose 1:1. D. lysozyme–trehalose 1:1. E. lysozyme–sucrose–dextran 5:3.5:1.5. F. lysozyme–sucrose–dextran 5:1.5:3.5.

increasing amounts of dextran, but was increased as trehalose replaced the sucrose. The differences in the effects of the sucrose–dextran content on the Tgs of the resultant powders produced by the freeze-drying and spray-drying processes are likely to primarily arise from the differences in the moisture content of the freeze- and spray-dried powders. For freeze-dried powders, all formulations contained a similar moisture content, which was independent of the composition. However, the moisture content of spray-dried combinations of sucrose with either lysozyme or lysozyme and dextran was found to be inversely related to the content of the former (Liao et al 2002). It has been

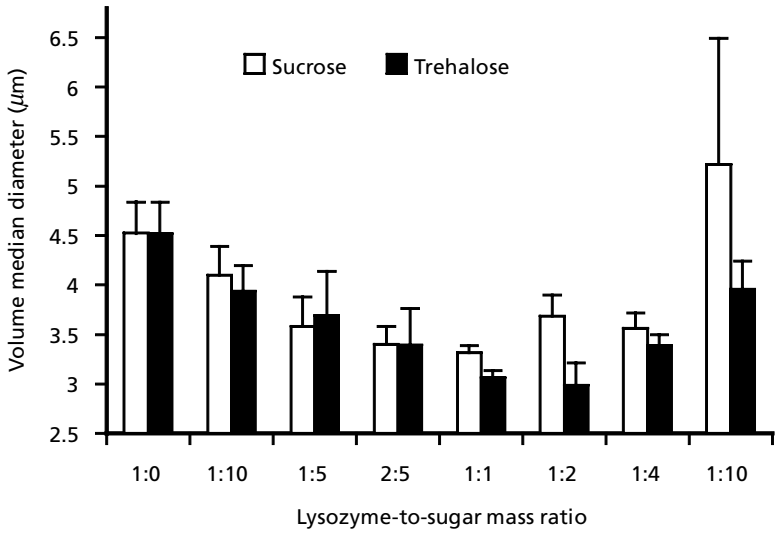

Figure 3 The effects of sucrose/trehalose on the particle size of spray-dried lysozyme–sucrose/trehalose powders (mean ± s.d., n = 3).

Table 3 Particle size distribution of spray-dried lysozyme formulations.

Lysozyme-to-sucrose-to-dextran mass ratio	Volume median diameter (μm)	Span
5:5:0	3.35 ± 0.14	0.93 ± 0.02
5:4:1	3.61 ± 0.36	1.00 ± 0.04
5:3.5:1.5	3.54 ± 0.32	0.96 ± 0.07
5:2.5:2.5	3.34 ± 0.16	0.87 ± 0.08
5:1.5:3.5	3.60 ± 0.12	1.02 ± 0.10
5:0:5	3.35 ± 0.26	0.88 ± 0.06

Data are means ± s.d., n = 3.

Table 4 Deposition results of formulations containing drug (spray-dried lysozyme–sucrose 1:1 and lysozyme–trehalose 1:1)-to-carrier mass ratio 1:6 within a twin-stage impinger after aerosolisation at 60 L mL^{-1} via a Rotahaler or glass inhaler.

Formulation	Inhaler	Device fraction (%)	Stage 1 fraction (%)	Stage 2 fraction (%)
Lysozyme–sucrose 1:1	Rotahaler	32.1 ± 5.7	45.1 ± 5.7	15.9 ± 2.4
	Glass inhaler	9.7 ± 3.7	47.5 ± 9.6	39.6 ± 6.9
Lysozyme–trehalose 1:1	Rotahaler	33.2 ± 3.7	40.9 ± 5.4	22.5 ± 2.0
	Glass inhaler	7.6 ± 1.0	47.6 ± 1.3	36.9 ± 0.3

Data are means ± s.d., n = 6.

well established that moisture has a plasticising effect on the formulation and lowers the Tg (Roos & Karel 1991; Shamblin & Zografi 1999) in accordance with the Gordon & Taylor equation (Gordon & Taylor 1952). Consequently, if the plasticising effects of water exceed the anti-plasticising functions of lysozyme and dextran, the Tg values of the formulation decrease. In contrast, the combining of trehalose with sucrose led to a minimal effect on the moisture content and the anti-plasticising effects dominated, resulting in a raised Tg. Therefore, the Tg of a spray-dried formulation could be raised by increasing the amount of trehalose, rather than dextran, or the model protein.

The glass of sucrose is thermodynamically unstable and it tends to transform to a more stable, but unwanted, crystalline form. Such a process involves nucleation and crystallisation, leading to a phase separation. In this study, the crystallisation of sucrose was found to be

inhibited by the presence of lysozyme in a concentration-dependent manner with the minimal content of lysozyme required to completely inhibit the crystallisation of spray-dried sucrose being ~33% w/w. In addition, when the lysozyme-to-sucrose mass ratio was at 1:1, the sucrose present in the spray-dried powder was found to resist crystallisation after storing at 58% r.h. These results suggest that the presence of sucrose in a protein formulation cannot exceed a certain mass ratio if the glassy sucrose is to be protected from crystallisation. Such inhibiting effects of lysozyme concurred with the findings from previous studies where the inhibitory capacity of somatotropin (Sarciaux & Hageman 1997), catalase, bovine pancreatic insulin and bovine pancreatic ribonuclease (Forbes et al 1998) has been reported. Somatotropin was found to increase the Tc of sucrose with increasing content up to 20% w/w (Sarciaux & Hageman 1997), whereas the other proteins were shown to inhibit the crystallisation of spray-dried lactose-containing powders.

In this study, spray-drying was demonstrated to be a feasible process to produce fine particles, independent of the excipient(s) utilised. In terms of geometric particle size, most of the spray-dried formulations appeared to contain particles, 95% of which were $< 5\,\mu$m as indicated by laser diffraction. The particle size was found to be dependent on excipient and concentration, although the scanning electron micrographs showed qualitatively that there were no gross differences in particle morphology with varying excipients. The deposition data indicated that spray-dried particles produced an FPF of up to 40%, and thus the powders would appear to be highly respirable in general. In addition, the difference in the respirable fraction of sucrose- and trehalose-containing powders appeared to be minimal when a glass inhaler device was utilised.

Conclusion

The physical properties of spray-dried formulations were found to be dependent upon composition, with trehalose-containing formulations being superior to the equivalent sucrose-based analogues in terms of the higher Tgs of the former. In addition, the Tg of the spray-dried particles appeared to increase with increasing sugar content in the formulations. Such a trend broadly corresponded to the degree of stabilisation conferred by sugars to spray-dried lysozyme. However, sucrose was found to be more effective than trehalose in preserving the native structure of spray-dried lysozyme (Liao et al 2002). As a consequence of these findings, it would appear that, to optimise both the physical stability of the formulations and the protein stability, more than one excipient might be required. For example, if the inclusion of sucrose is necessary for the higher stabilising capacity it confers relative to trehalose, the physical stability could be raised by the inclusion of trehalose, albeit not dextran. In this study, spray-drying was suitable to produce fine particles, the morphology of which seemed to be independent of excipient(s). The sugar-to-lysozyme mass ratio required to achieve a minimum geometric particle size appeared to be between 1:1 and 2:1. Coincidentally, the physical stability of the resultant particles and the stabilisation of lysozyme were close to the maximum at such mass ratios. Thus, spray-drying could be a feasible means producing respirable particles, which would enable protein integrity to be maintained and suitable physical stability to be conferred providing the excipients were carefully selected and were present at an appropriate concentration.

References

Adjei, A., Garren, J. (1990) Pulmonary delivery of peptide drugs: effects of particle size on bioavailability of leuprolide acetate in healthy human male volunteers. *Pharm. Res.* **7**: 565–569

Adler, M., Lee, G. (1999) Stability and surface activity of lactate dehydrogenase in spray-dried trehalose. *J. Pharm. Sci.* **88**: 199–208

Allison, S. D., Manning, M. C., Randolph, T. W., Middleton, K., Davis, A., Carpenter, J. F. (2000) Optimisation of storage stability of lyophilised Actin using combinations of disaccharides and dextran. *J. Pharm. Sci.* **89**: 199–214

Andya, J. D., Maa, Y. F., Costantino, H. R., Nguyen, P. A., Dasovich, N., Sweeney, T. D., Hsu, C. C., Shire, S. J. (1999) The effect of formulation excipients on protein stability and aerosol performance of spray-dried powders of a recombinant humanised anti-IgE monoclonal antibody. *Pharm. Res.* **16**: 350–358

Bosquillon, C., Lombry, C., Preat, V., Vanbever, R. (2001) Influence of formulation excipients and physical characteristics of inhalation dry powders on their aerosolisation performance. *J. Control. Release* **70**: 329–339

Branchu, S., Forbes, R. T., York, P., Petren, S., Nyqvist, H., Camber, O. (1999) Hydroxypropyl-beta-cyclodextrin inhibits spray-drying-induced inactivation of beta-galactosidase. *J. Pharm. Sci.* **88**: 905–911

Broadhead, J., Rouan, S., Hau, I., Rhodes, C. T. (1994) The effect of process and formulation variables on the properties of spray dried β-galactosidase. *J. Pharm. Pharmacol.* **46**: 458–467

Chan, H. K., Gonda, I. (1998) Solid state characterisation of spray-dried powders of recombinant human deoxyribonuclease (RhDNase). *J. Pharm. Sci.* **87**: 647–654

Chan, H. K., Clark, A., Gonda, I., Mumenthaler, M., Hsu, C. (1997) Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. *Pharm. Res.* **14**: 431–437

Chang, B. S., Beauvais, R. M., Dong, A., Carpenter, J. F. (1996) Physical factors affecting the storage stability of freeze-dried Interleukin-1 receptor antagonist: Glass transition and protein conformation. *Arch. Biochem. Biophys.* **331**: 249–258

Cleland, J. L., Daugherty, A., Mrsny, R. (2001) Emerging protein delivery methods. *Curr. Opin. Biotech.* **12**: 212–219

Costantino, H. R., Andya, J. D., Nguyen, P. A., Dasovich, N., Sweeney, T. D., Shire, S. J., Hsu, C. C., Maa, Y. F. (1998) Effect of mannitol crystallisation on the stability and aerosol performance of a spray-dried pharmaceutical protein, recombinant humanised anti-IgE monoclonal antibody. *J. Pharm. Sci.* **87**: 1406–1411

Crowe, J. H., Carpenter, J. F., Crowe, L. M. (1998) The role of vitrification in anhydrobiosis. *Annu. Rev. Physiol.* **60**: 73–103

Forbes, R. T., Davis, K. G., Hindle, M., Clarke, J. G., Maas, J. (1998) Water vapor sorption studies on the physical stability of

a series of spray-dried protein/sugar powders for inhalation. *J. Pharm. Sci.* **87**: 316–321

Gordon, M., Taylor, J. S. (1952) Ideal copolymers and the second-order transitions of synthetic rubbers, I: non-crystalline copolymer. *J. Appl. Chem.* **2**: 493–498

Labrude, P., Rasolomanana, M., Vigneron, C., Thirion, C., Chaillot, B. (1989) Protective effect of sucrose on spray drying of oxyhemoglobin. *J. Pharm. Sci.* **78**: 223–229

Liao, Y. H., Brown, M. B., Martin, G. P. (2001) Turbidimetric and HPLC assays for the determination of formulated lysozyme activity. *J. Pharm. Pharmcol.* **53**: 549–554

Liao, Y. H., Brown, M. B., Nazir, T., Quader, A., Martin, G. P. (2002) Effects of sucrose and trehalose on the preservation of the native structure of spray-dried lysozyme. *Pharm. Res.* **19**: 1849–1855

Lopez-Diez, E. C., Bone, S. (2000) An investigation of the water-binding properties of protein and sugar systems. *Phys. Med. Biol.* **45**: 3577–3588

Maa, Y. F., Nguyen, P. A. T., Hsu, S. W. (1998a) Spray-drying of air-liquid interface sensitive recombinant human growth hormone. *J. Pharm. Sci.* **87**: 152–159

Maa, Y. F., Nguyen, P. A., Andya, J. D., Dasovich, N., Sweeney, T. D., Shire, J., Hsu, C. C. (1998b) Effect of spray drying and subsequent processing conditions on residual moisture content and physical/biochemical stability of protein inhalation powders. *Pharm. Res.* **15**: 768–775

MacRitchie, H. B. (1998) *The development of a laser diffraction technique for use in the physical characterisation of dry powders for inhalation*. Ph. D. Thesis, University of London

Murray, B. S., Liang, H. J. (1999) Enhancement of the foaming properties of protein dried in the presence of trehalose. *J. Agric. Food Chem.* **47**: 4984–4991

Roos, Y., Karel, M. (1991) Phase-transitions of amorphous sucrose and frozen sucrose solutions. *J. Food Sci.* **56**: 266–267

Saleki-Gerhardt, A., Zografi, G. (1994) Non-isothermal and isothermal crystallization of sucrose from the amorphous state. *Pharm. Res.* **11**: 1166–1173

Sarciaux, J. M. E., Hageman, M. J. (1997) Effects of bovine somatotropin (rbSt) concentration at different moisture levels on the physical stability of sucrose in freeze-dried rbSt/Sucrose mixtures. *J. Pharm. Sci.* **86**: 365–371

Shamblin, S. L., Zografi, G. (1999) The effects of absorbed water on the properties of amorphous mixtures containing sucrose. *Pharm. Res.* **16**: 1119–1124

Slade, L., Levine, H. (1988) Non-equilibrium behaviour of small carbohydrate water system. *Pure Appl. Chem.* **60**: 1841–1864

Tzannis, S. T., Prestrelski, S. J. (1999) Activity-stability considerations of trypsinogen during spray drying: effects of sucrose. *J. Pharm. Sci.* **88**: 351–359

Wearley, L. L. (1991) Recent progress in protein and peptide delivery by noninvasive routes. *Crit. Rev. Ther. Drug* **8**: 331–394