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Novel pMDI formulations for pulmonary delivery of proteins

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ABSTRACT

In this paper, we demonstrate that co-spray-drying a model protein with sodium carboxymethylcellulose (NaCMC) protects protein integrity during spray-drying, and that the resultant spray-dried powders can be successfully dispersed in hydrofluoroalkane (HFA) propellant to prepare pressurised metered dose (pMDI) formulations that exhibit high respirable fractions. The spray-dried powders were formulated as HFA-134a pMDI suspensions in the absence of any other excipients (e.g. surfactants) or co-solvents (e.g. ethanol). The *in vitro* aerosolisation profile of these systems was assessed using the twin stage impinger; fine particle fractions (FPF) ≥50% of the recovered dose were obtained. Following storage for five months, the aerosolisation performance was reassessed; the NaCMC-free formulation demonstrated a significant decrease in FPF, whereas the performance of the NaCMC-modified formulations was statistically equivalent to their initial performance. Thus, formulation of pMDI suspensions using NaCMC-based spray-dried powders is a promising approach for the pulmonary delivery of proteins and peptides.

1. Introduction

The relatively large alveolar surface area (approximately 150 m^2), thin alveolar epithelium (0.1–0.5 μm) and avoidance of first pass metabolism make pulmonary administration an attractive delivery route for systemically acting drugs that are not suitable for delivery via the oral route, for example insulin and other protein and peptide macromolecules, and also more novel biotechnological agents such as gene therapy and vaccines (Yu and Chien, 1997; Codrons et al., 2003; Jenkins et al., 2003). A number of groups in the field of pulmonary drug delivery have existing investigations into the delivery of proteins and peptides to the lung, most notably insulin for the treatment of diabetes. However, the majority of research is aimed at producing respirable dry powders to facilitate the development of dry powder inhaler (DPI) devices, with some investigators considering the use of nebulisation to deliver proteins to the lung (Johnson, 1997; Sangwan et al., 2001; Sinah and Trehan, 2003; Steckel et al., 2003). Fewer investigations appear to have been published on the development of pressurised metered dose inhaler (pMDI) systems for the pulmonary delivery of macromolecules (Brown and George, 1997; Williams and Liu, 1999; Nakate et al., 2003; Myrdal et al., 2004; Hausmann et al., 2006). Nevertheless, research has shown that of all the pulmonary drug delivery devices available for use by patients, the pMDI is the device of choice, being well accepted by both patients and clinicians due to its relative low cost, portability and disposability (Boyd, 1995; Terzano, 2001; Garcia-Contreras and Smyth, 2005; Shoyele and Slowey, 2006).

Most drugs, including proteins and peptides, exhibit negligible solubility in the hydrofluoroalkane (HFA) propellants used in the preparation of pMDI systems, therefore suspension formulations, employing micronised drug powders, are required. Micronisation is traditionally accomplished using mechanical methods such as jet milling, where resultant particles typically have an irregular morphology and wide size distribution. Surfactants are usually added to pMDI formulations to improve the dispersibility of the particles in the propellant (Williams and Liu, 1999; Williams et al., 1999). Alternative methods of particle size reduction include spray-drying, with many researchers demonstrating that this method generates particles of a respirable size with good aerosolisation properties (Seville et al., 2007b; Adi et al., 2008; Learoyd et al., 2008; Lechuga-Ballesteros et al., 2008). Although this technology has been widely used in the preparation of powders for inhalation from a DPI, it has apparently been employed rarely in the preparation of pMDI formulations (Tarara et al., 2004; Jones et al., 2006a,b). A major concern with the development of pMDI formulations of proteins and peptides is that the propellant might denature the protein; in order to maintain the biological activity of the protein, it is necessary to preserve its three-dimensional conformation whilst dispersed in the propellant and during the aerosolisation process (Byron, 1990; Shoyele and Slowey, 2006; Engstrom et al., 2009). It is notable, however, that several proteins (e.g. lysozyme) have demonstrated reasonable stability when formulated as suspension pMDI systems (Quinn et al., 1999).

In an attempt to preserve the three-dimensional conformation of the protein (a) during the spray-drying process and (b) dur-

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ing the preparation and aerosolisation of the pMDI device, we investigated the use of co-spray-drying the protein with sodium carboxymethylcellulose (NaCMC). This water-dispersible polysaccharide, generally regarded as non-toxic and non-irritant, is widely used in oral, topical and some parenteral formulations as a viscosity modifier, a binder and disintegrant in tableting and an emulsion stabiliser (Parsons, 2000). NaCMC has also been investigated for its drug release (Rokhade et al., 2006) and mucoadhesive (Ludwig, 2005) properties. Although a few groups have prepared NaCMC by spray-drying (Wan et al., 1993; Law and Deasy, 1998; Billon et al., 2000), as far as we are aware no-one has considered the use of NaCMC-based spray-dried powders in the preparation of pulmonary delivery systems. In this paper, we use spray-dried powders comprising bovine serum albumin (BSA) as a model protein and differing amounts of NaCMC to prepare surfactant-free pMDI suspension formulations.

2. Materials and methods

2.1. Materials

Bovine serum albumin (BSA, molecular weight 66 kDa) was obtained from Sigma–Aldrich Chemicals (Poole, UK). Sodium carboxymethylcellulose (NaCMC) was obtained from Aqualon (Hercules GmbH, Düsseldorf, Germany: Blanose®). Hydrofluoroalkane (HFA)-134a (pharmaceutical grade) was purchased from INEOS Fluor (Cheshire, UK). HPLC-grade water, HPLC-grade acetonitrile, trifluoroacetic acid, and other all chemicals were purchased from Fisher Scientific Ltd. (Loughborough, UK).

2.2. Preparation of spray-dried powders

Spray-drying feedstocks were prepared by fully dissolving 200 mg BSA and an appropriate quantity of NaCMC (100–800 mg) in 20 mL double distilled water to give final concentrations of 1% (w/v) BSA and 0.5–4% (w/v) NaCMC. Each feedstock was prepared in triplicate.

Prior to spray-drying, the viscosity of the feedstocks was assessed using an AMVn automated microviscometer (Anton Parr, Graz, Austria) with a 1.6 mm diameter capillary, pre-calibrated using double distilled water with a capillary inclination of 20–90° and a temperature of 20 °C. Feedstock viscosity was determined at a capillary inclination of 70 °C and a temperature of 20 °C; each sample was measured four times.

The prepared feedstocks were subsequently spray-dried using a Büchi B-290 mini spray-dryer equipped with a high performance cyclone (Büchi Labortechnik AG, Switzerland), using the following standard operating conditions: aspirator setting 85% (34 m^3/h); spray-flow rate 600 L/h; inlet temperature 180 °C; outlet temperature 80 °C. Following spray-drying, the powders were collected from the lower part of the cyclone and the collecting vessel, stored in tightly sealed glass vials and the spray-drying yield noted. A total of 6 spray-dried powders were generated ($n = 3$); the control powder nominally comprised 100% (w/w) BSA, whereas the NaCMC-modified powders contained 20–67% (w/w) BSA and 33–80% (w/w) NaCMC.

2.3. Powder characterisation

The moisture content of the spray-dried powders was determined using thermogravimetric analysis (Pyris 1 TGA: PerkinElmer, Waltham, USA). Samples (5 mg) were placed into platinum pans and analysed under a nitrogen purge (20 mL min^{-1}) over the temperature range 40–140 °C at a heating rate of 10 °C per minute. Measurements were performed in triplicate.

Laser diffraction (HELOS particle size analyser: Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, Germany) was used to determine the particle size distribution of the spray-dried powders. The sample dispersion system used was the VIBRO/RODOS dry dispersion system, and a pressure of 1 bar was employed to disperse 100 mg of each powder to achieve the required obscuration of 5%. Samples were measured in triplicate.

Selected spray-dried powders were sputter coated with a thin layer of gold using an EMScope sputter-coater under partial vacuum and representative images obtained using a Philips XL30 scanning electron micrograph.

2.4. Preparation of pMDI formulations

pMDI formulations of the spray-dried powders were prepared by adding 100 mg of the powder into pre-weighed plastic pMDI vials. A BK357 30 μL valve was crimped onto the vials using a Pamasol P2002 small scale crimper and approximately 6 g HFA-134a was pressure-filled through the valve using a Pamasol P2011 propellant filler (Pamasol Willi Mäder AG, Pfäffikon, Switzerland) to form suspensions with an approximate concentration of 1.7% (w/w) powder in propellant. The formulations were vortex-mixed for 1 min then stored at room temperature, valves up.

2.4.1. In vitro powder aerosolisation

The aerosolisation performance of the pMDI formulations was determined using the twin stage impinger (TSI: Copley Scientific Ltd., Nottingham, UK) in accordance with the British Pharmacopoeia specifications. Double distilled water was introduced into the upper and lower stages (7 and 30 mL, respectively), and the flow rate through the TSI adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific). The pMDI formulations were primed prior to use by actuating 5 shots to waste. A total of 20 actuations were sprayed into the TSI from each pMDI. The pump was allowed to run for 5 s after each discharge and then switched off for 5 s whilst the inhaler was shaken by hand. After the completion of each run, the impinger was dismantled and the amount of BSA deposited in Stage 1 and Stage 2 of the TSI was determined. In addition, the pMDI actuator and the TSI throat were each rinsed with 5 mL of double distilled water and these fractions analysed for BSA content. The pMDI vial was weighed before and after each run, to enable the emitted dose (ED) of BSA per actuation to be calculated, based on the proportion of BSA in each formulation. Each aerosolisation test was performed in triplicate. The recovered dose (RD) was defined as the total mass of BSA detected (i.e. actuator + throat + Stage 1 + Stage 2) expressed as the percentage of ED. The fine particle dose (FPD) was defined as the mass of BSA recovered from Stage 2 of the TSI (effective cut-off diameter 6.4 μm). The fine particle fraction (FPF) was calculated as the ratio of FPD to RD, expressed as a percentage.

2.4.2. pMDI stability

In order to obtain a preliminary assessment of the stability of the various formulations, the pMDI suspensions were shaken by hand for 5 s then left to settle for two hours. The uniformity of the suspensions was evaluated by eye at various time intervals, and the ease of redispersion at the end of two hours determined by re-shaking for 5 s. In addition, the pMDI formulations were stored at room temperature for five months, after which time the aerosolisation performance of the formulations was reassessed.

2.5. Quantification of BSA

The mass of BSA in each spray-dried powder and the amount deposited in the TSI following aerosolisation of selected powders

was quantified using reverse-phase HPLC (Dionex AS50 autosampler with GP50 Gradient pump HPLC System: Dionex, Surrey, UK) at room temperature using a 4.6 mm × 250 mm Jupiter 5 μm C18/ODS 300 Å column (Phenomenex, Torrance, USA) and a 50 μL injection volume with UV detection at 280 nm and gradient elution employing two solutions: solution A (0.1%, v/v trifluoroacetic acid in water) and solution B (0.1%, v/v trifluoroacetic acid in acetonitrile). A flow rate of 1 mL/min was employed and the column was eluted over 20 min per sample, during which time the proportion of solution B in the eluant increased from 25% to 65% (Phenomenex, 2007). BSA eluted with a retention time of 12.2 min. A calibration curve was constructed using BSA standard solutions of 100–500 μg/mL (r^2 = 0.9986) against which the concentration of BSA in unknown solutions was determined. Each sample was measured in triplicate.

3. Results and discussion

3.1. Feedstock viscosity

Increasing the amount of NaCMC added to the spray-drying feedstocks from 0% (w/v) to 4% (w/v) resulted in a linear increase in the viscosity of the feedstock ($y = 9.2981x + 0.7316$, $r^2 = 0.9987$). The viscosity of a solution containing 1% (w/v) BSA (i.e. NaCMC-free) was found to be 1.0 mPa s. Addition of 0.5% (w/v) NaCMC to the feedstock increased the viscosity to 5.3 mPa s, whereas a viscosity of 38.5 mPa s was measured for a solution containing 0.5% (w/v) BSA and 4% (w/v) NaCMC. It was not feasible to spray-dry formulations containing >4% (w/v) NaCMC due to the high viscosity of the feedstock.

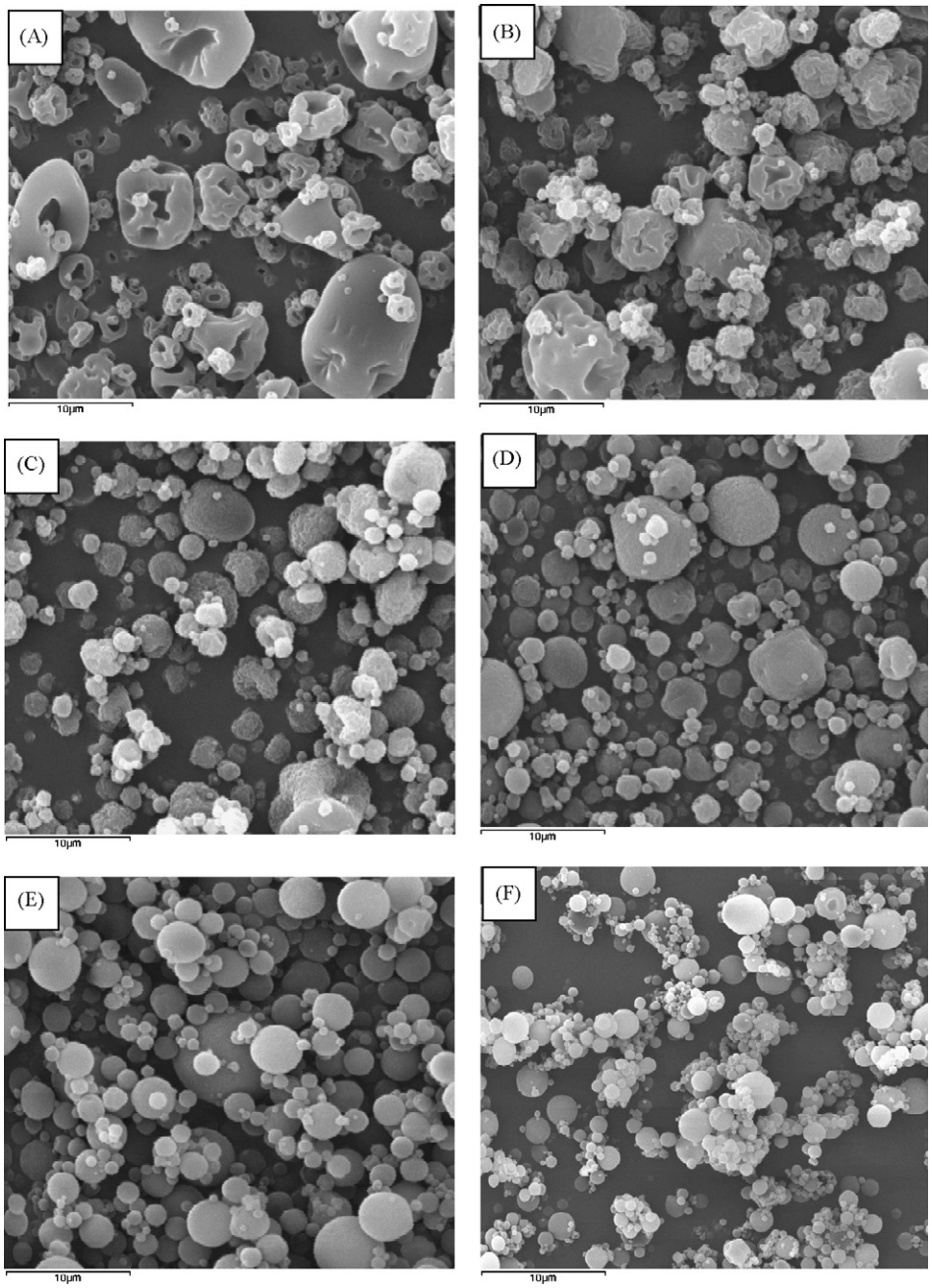

Fig. 1. Scanning electron micrographs of spray-dried powders: (A) 100% (w/w) BSA; (B) 67% (w/w) BSA–33% (w/w) NaCMC; (C) 50% (w/w) BSA–50% (w/w) NaCMC; (D) 33% (w/w) BSA–67% (w/w) NaCMC; (E) 25% (w/w) BSA–75% (w/w) NaCMC; (F) 20% (w/w) BSA–80% (w/w) NaCMC.

Table 1
Physical properties of the spray-dried powders (mean ± SD, $n = 3$).

Powder	Yield (%)	Moisture content (%)	BSA content (%)
0% (w/w) NaCMC	57.0 ± 1.3	5.5 ± 0.6	71.2 ± 3.3
33% (w/w) NaCMC	63.3 ± 1.3‡	7.4 ± 1.0†	99.4 ± 0.5‡
50% (w/w) NaCMC	62.7 ± 1.1‡	7.8 ± 0.8‡	97.4 ± 3.2‡
67% (w/w) NaCMC	60.6 ± 1.4‡	8.5 ± 0.5‡	98.6 ± 2.7‡
75% (w/w) NaCMC	58.8 ± 0.6	9.0 ± 0.2‡	97.7 ± 2.1‡
80% (w/w) NaCMC	57.8 ± 0.9	9.4 ± 0.4‡	100.8 ± 2.2‡

† $p < 0.05$ statistical difference (one-way ANOVA/Dunnett) from control.
‡ $p < 0.01$ statistical difference (one-way ANOVA/Dunnett) from control.

3.2. Spray-dried powder characteristics

The yield, moisture content and BSA content of the spray-dried powders are presented in Table 1. Spray-drying BSA alone resulted in a reasonable yield (57%), however substantial degradation of BSA occurred during the spray-drying process, presumably as a consequence of the thermal instability of the protein, resulting in this powder containing only 71% of the expected amount of BSA, as quantified using HPLC. Spray-drying the NaCMC-modified powders also resulted in reasonable yields (58–63%), however inclusion of this excipient in the spray-drying feedstock resulted in a significant increase BSA content of the spray-dried powders (one-way ANOVA with Dunnett multiple comparisons test, $p < 0.01$), with approximately 100% of the anticipated amount of BSA detected in the NaCMC-modified spray-dried powders. This suggests that incorporation of NaCMC protected the BSA from thermal degradation during the spray-drying process. When added to water, NaCMC disperses to form a colloidal solution (Parsons, 2000), and it is feasible that the BSA is entrapped within these colloids, and thereby protected from degradation during spray-drying, as has previously been suggested for other spray-drying excipients such as leucine (Rabbani and Seville, 2005). The moisture content of the BSA spray-dried powder was 5.5%, in line with the moisture content observed by other researchers investigating spray-dried BSA (e.g. Chew and Chan, 2001). The addition of NaCMC to the spray-drying feedstock resulted in a significant ($p < 0.01$) increase in the moisture content of the resultant spray-dried powders compared to the NaCMC-free powder, with higher moisture contents observed in powders containing a greater proportion of NaCMC.

Scanning electron microscopy (Fig. 1) indicated that the BSA spray-dried powder (Fig. 1a) comprised particles of an irregular shape with indentations on the surface, suggesting the appearance of a collapsed sphere. A similar morphology for spray-dried BSA particles was recently observed by Engstrom et al. (2009), who described their particles as having a "corrugated" surface. Similar morphologies were also apparent in the NaCMC powders (Fig. 1b–f), although it was noted that fewer deflated spheres were present in the images at higher NaCMC concentrations, with particles taking on a more rounded appearance. Fig. 2 shows the particle size distribution of the spray-dried powders. The median and mean diameters of the BSA and NaCMC-modified powders were comparable (2.7–3.4 and 3.6–4.3 μm, respectively), indicating that the addition of NaCMC to the spray-drying feedstock did not increase the size of the droplet formed at the spray-drying nozzle. All powders were of a suitable size for pulmonary delivery.

3.3. Aerosolisation performance

The spray-dried powders were formulated as pMDI suspensions, using HFA-134a as the propellant. All powders dispersed well within the propellant, with no aggregates visible to the naked eye. Fig. 3 displays the deposition profile following aerosolisation of 20 actuations from each formulation into the TSI. Encouragingly, all formulations exhibited high deposition in Stage 2 of the

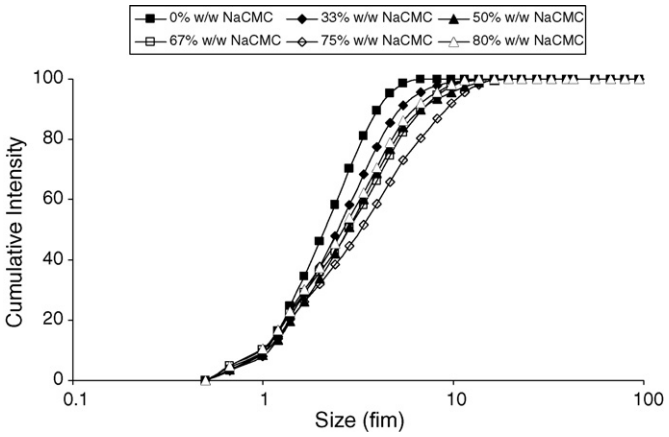

Fig. 2. Particle size distribution of spray-dried powders.

TSI (effective cut-off diameter 6.8 μm), with minimal deposition on the pMDI actuator and Stage 1 of the TSI. In each formulation, the FPF (Table 2) was in excess of 45% of the total recovered dose, suggesting that each of these formulations would be predicted to demonstrate high deposition in the lower respiratory tract. The 75% (w/w) NaCMC formulation demonstrated statistically ($p < 0.05$) higher FPF compared to the NaCMC-free formulation (54% versus 46%) and lower deposition in the throat (22% versus 30%), whereas all other NaCMC-modified formulations demonstrated equivalent aerosolisation performance to the NaCMC-free powder. The recovered dose for each pMDI formulation was greater than 95% of the anticipated amount (corrected for BSA content in each powder).

The rate of sedimentation of the pMDI suspensions was investigated by shaking the pMDI formulations by hand, and leaving them to settle for two hours, over which time photographs of the formulations were taken (Fig. 4). It was noted that the 33% (w/w) and the 50% (w/w) NaCMC spray-dried powders sank rapidly, whereas the 75% (w/w) and the 80% (w/w) NaCMC powders floated in the propellant. Interestingly, the 67% (w/w) NaCMC powder remained relatively well dispersed throughout the propellant over the two-hour test period. All formulations easily redispersed with gentle shaking at the end of the test, and indeed following storage for five months at room temperature. Although no-one has investigated the use of NaCMC as a suspending agent for the preparation of pMDI formulations, it has been used in the preparation of aqueous suspensions intended for oral administration (Zietsman et al., 2007; Junyaprasert and Manwiwattanakul, 2008). It is likely that the pres-

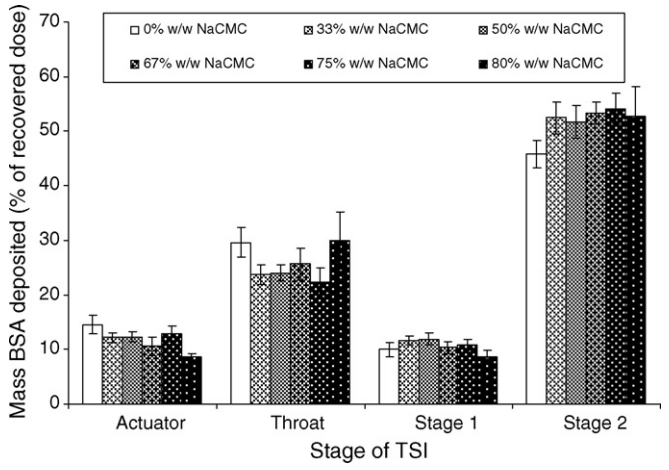

Fig. 3. Deposition profile of the pMDI formulations following aerosolisation into the twin stage impinger (mean ± SD, $n = 3$).

Table 2
Aerosolisation properties of the pMDI formulations (mean ± SD, $n = 3$) immediately after preparation and following storage for five months.

Powder	ED (μg)	RD initially (%)	FPD initially (μg)	FPF initially (% of RD)	RD after storage (%)	FPF after storage (% of RD)
0% (w/w) NaCMC	368	98.8 ± 3.6	167 ± 15	46 ± 2	80.1 ± 5.9	35 ± 2
33% (w/w) NaCMC	339	101.7 ± 1.2	181 ± 10	52 ± 3	96.8 ± 3.5	48 ± 2‡
50% (w/w) NaCMC	239	99.8 ± 3.6	123 ± 10	52 ± 3	98.9 ± 2.2	50 ± 2‡
67% (w/w) NaCMC	165	101.5 ± 6.1	90 ± 5	53 ± 2	100.1 ± 2.1	55 ± 2‡
75% (w/w) NaCMC	120	100.7 ± 3.0	66 ± 5	54 ± 3†	99.0 ± 2.7	49 ± 2‡
80% (w/w) NaCMC	102	98.0 ± 5.9	53 ± 8	53 ± 5	97.0 ± 3.0	48 ± 3‡

ED: emitted dose per actuation; RD: recovered dose; FPD: fine particle dose; FPF: fine particle fraction.
† $p < 0.05$ statistical difference (one-way ANOVA/Dunnett) in FPF from control.
‡ $p < 0.01$ statistical difference (one-way ANOVA/Dunnett) in FPF from control.

ence of NaCMC on the surface of the particles acts as a polymeric flocculating agent, thereby stabilising the suspension.

The aerosolisation properties of the pMDI formulations were reassessed following storage of the canisters for five months.

Fig. 4. Settling behaviour of the pMDI formulations at (I) 15 s; (II) 45 s; (III) 10 min; and (IV) 2 h after manual shaking: (A) 100% (w/w) BSA; (B) 67% (w/w) BSA–33% (w/w) NaCMC; (C) 50% (w/w) BSA–50% (w/w) NaCMC; (D) 33% (w/w) BSA–67% (w/w) NaCMC; (E) 25% (w/w) BSA–75% (w/w) NaCMC; (F) 20% (w/w) BSA–80% (w/w) NaCMC.

Although the BSA formulation had exhibited a reasonable FPF immediately after preparation (46%), the FPF following storage had decreased to only 35% (Table 2). In contrast, the NaCMC-modified spray-dried powders demonstrated statistically equivalent performance to their original aerosolisation performance, and statistically higher FPF than the BSA formulation ($p < 0.01$). Furthermore, the 67% (w/w) NaCMC formulation demonstrated statistically (one-way ANOVA with Tukey–Kramer multiple comparisons test, $p < 0.05$) higher FPF than the 33% (w/w) NaCMC and 80% (w/w) NaCMC formulations. The total recovered dose for the BSA formulation following storage was only 80% of the anticipated amount, suggesting that some of the BSA had adsorbed to the pMDI canister walls, effectively removing it from the aerosolised dose, or that the BSA may have been denatured by the propellant over the storage period (Shoyele and Slowey, 2006). The total recovered dose for the NaCMC formulations following storage was greater than 95% of the anticipated amount, suggesting that NaCMC either prevented adsorption of BSA to the canister walls or prevented degradation of BSA over the storage period.

Our approach to preserve the three-dimensional conformation of the protein during the spray-drying process and whilst in the pMDI device is to co-spray dry the protein in the presence of suitable excipients, thereby forming a composite particle in which the protein is entrapped and potentially protected from external conditions. Williams and Liu (1999) considered a similar approach, employing lyophilisation to generate composite particles of BSA and surfactant. Dispersion of these surfactant/protein powders in HFA-134a/ethanol generated stable, flocculated suspensions that were capable of generating high respirable fractions, with the best performance obtained from a system comprising a 500:1 molar ratio of Tween 80:BSA suspended in a propellant system comprising 92% HFA-134a, 8% ethanol. However, the amount of surfactant required and the need for ethanol as a co-solvent raise concerns over the potential toxicity of such a system. Furthermore, the high surfactant/protein ratio results in an extremely low dose of protein per actuation.

Liao et al. (2005) recently demonstrated that spray-drying lysozyme in the presence of polyvinyl alcohol (PVA) and/or trehalose preserved the biological activity of the enzyme compared to spray-drying the enzyme alone. They found that a spray-dried lysozyme/trehalose formulation demonstrated relatively poor aerosolisation performance, with an FPF of less than 30%, decreasing to 8% on storage, whereas a spray-dried lysozyme/trehalose/PVA formulation demonstrated a significantly higher fine particle fraction (>50%) compared to the PVA-free formulation; this aerosolisation performance appeared to be preserved on storage. The authors attributed this to the inclusion of the polymeric surfactant PVA, which was thought to result in reduced particle agglomeration arising from preferential adsorption of the surfactant at the surface of the droplets during the spray-drying procedure.

In contrast with the previously reported studies, the pMDI protein formulations reported in this paper are surfactant-free, solvent-free, highly stable suspensions of spray-dried proteins that

exhibit excellent aerosolisation performance that is maintained during storage. Co-spray-drying proteins and peptides with NaCMC may therefore offer an alternative method for the preparation of stable and respirable pMDI formulations for pulmonary delivery. In particular, the 67% (w/w) NaCMC formulation appeared to offer the optimal pMDI characteristics is terms of ease of redispersion on shaking and maintenance of fine particle fraction following storage.

4. Conclusions

We have established that spray-drying sodium carboxymethylcellulose-based feedstocks generates dry powders that can be successfully formulated as suspension pMDI systems for the delivery of BSA without the need for additional excipients such as suspending agents or co-solvents. These novel systems demonstrate promise for the systemic delivery of macromolecules, and further studies are ongoing with other, functional, proteins (e.g. alkaline phosphatise, β-galactosidase and catalase) to investigate whether other proteins and peptides can also be successfully formulated using this approach. For instance, the incorporation of aerosolisation enhancers such as leucine to the spray-drying feedstock has been shown to be a useful method of improving the deposition profile of dry powders for inhalation (Seville et al., 2007a); it would be interesting to consider whether leucine would elicit a similar effect when included in NaCMC-based spray-dried powders formulated as pMDI suspensions.

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