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Research paper

Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate

Tristan P. Learoyd [a], Jane L. Burrows [b], Eddie French [b], Peter C. Seville [a,*]

[a] School of Life and Health Sciences, Aston University, Birmingham, UK
[b] Pfizer Global Research and Development, Kent, UK

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Abstract

In this study, we describe the preparation of highly dispersible dry powders for pulmonary drug delivery that display sustained drug release characteristics. Powders were prepared by spray-drying 30% v/v aqueous ethanol formulations containing terbutaline sulfate as a model drug, chitosan as a drug release modifier and leucine as an aerosolisation enhancer. The influence of chitosan molecular weight on the drug release profile was investigated by using low, medium and high molecular weight chitosan or combinations thereof. Following spray-drying, resultant powders were characterised using scanning electron microscopy, laser diffraction, tapped density analysis, differential scanning calorimetry and thermogravitational analysis. The in vitro aerosolisation performance and drug release profile were investigated using Multi-Stage Liquid Impinger analysis and modified USP II dissolution apparatus, respectively. The powders generated were of a suitable aerodynamic size for inhalation, had low moisture content and were amorphous in nature. The powders were highly dispersible, with emitted doses of over 90% and fine particle fractions of up to 82% of the total loaded dose, and mass median aerodynamic diameters of less than 2.5 μm. A sustained drug release profile was observed during dissolution testing; increasing the molecular weight of the chitosan in the formulation increased the duration of drug release.

Keywords: Spray-drying; Leucine; Modified release; Inhalation; Aerosolisation; Chitosan

1. Introduction

Inhalation therapy is widely employed to deliver drugs to the respiratory epithelium, predominately for the treatment of local disorders such as asthma and COPD, although there is increasing interest in using pulmonary delivery for the administration of systemically-acting macromolecules, exemplified most notably by the recent launch of the inhaled insulin product, Exubera [1].

When formulating a dry powder for inhalation, micronisation is usually employed to reduce the particle size of the drug powder to less than 5 μm. However, powders in this size range exhibit strong interparticulate cohesion, leading to poor powder flow properties [2,3]. Furthermore, factors known to influence the aerosolisation properties of dry powders (e.g. particle morphology, density and surface composition [4]) cannot be controlled effectively during the micronisation process. Researchers in the field have investigated a number of approaches to improve powder aerosolisation, such as mixing the micronised drug with inert carrier particles [5–8] or modification of particle morphology [9,10], particle surface roughness [11], particle porosity [12] or powder density [2,13]. An alternative approach to the generation of dry powders for pulmonary drug delivery is offered by spray-drying technology. Whereas micronisation is a destructive technique, spray-drying is a one-step constructive process that provides greater control over particle size, particle morphology and powder density. Indeed, dry powders generated by

* Corresponding author. Inhalation Technology Research Team, School of Life and Health Sciences, Aston University, Birmingham B4 7ET, UK. Tel.: +44 212 204 4010; fax: +44 212 359 0733.
E-mail address: p.c.seville@aston.ac.uk (P.C. Seville).

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spray-drying have been investigated by a number of researchers for suitability as dry powder inhaler (DPI) formulations (e.g. [12,14–19]).

Spray-drying technology also offers the potential to incorporate a range of excipients into the formulation to be spray-dried, including dispersibility enhancers (e.g. leucine [20–22]) to modify the aerosolisation characteristics of the resultant powder. In addition, spray-dried powders that exhibit sustained drug release properties may be generated through the inclusion of drug release modifiers such as hydroxypropyl cellulose [23], glyceryl behenate [24] and polylactic acid [25]. Chitosan, a polysaccharide derived from deacetylation of the naturally occurring polymer chitin, is a promising excipient that can be employed in a wide range of applications, including sustained release preparations [26]. Indeed, this compound has received considerable attention for the formulation of spray-dried powders for nasal drug delivery (e.g. [27–33]).

There are many advantages to developing sustained release formulations for pulmonary drug delivery, including reduced dosing frequency, improved patient compliance and reduction in side effects [34]. Given that chitosan not only acts as a drug release modifier but also has mucoadhesive properties [32,33], it would appear to be a useful excipient when preparing sustained release formulations for pulmonary drug delivery. Although substantial research has been aimed at developing such formulations, only a handful of researchers have investigated the viability of chitosan-modified spray-dried powders for pulmonary drug delivery [35–38], with none apparently having considered the incorporation of dispersibility enhancers such as leucine to improve powder aerosolisation characteristics. This may be due to concerns surrounding the degradation of chitosan in the lung following administration and the potential for chitosan to elicit mild inflammatory responses [39]. Nevertheless, the observation that chitosan is less toxic than other polymers [40] suggests that it may be an interesting compound to investigate.

In this study, we demonstrate that spray-drying formulations of chitosan as a drug release modifier and leucine as a dispersibility enhancer generates highly dispersible powders that exhibit sustained drug release properties. We find that higher molecular weight chitosan powders exhibit somewhat poorer aerosolisation characteristics but substantially longer dissolution profiles compared to lower molecular weight chitosan formulations. We show that drug release from the chitosan powders follows square-root-time kinetics, and is dependent on the molecular weight of the chitosan. We demonstrate that by selecting an appropriate molecular weight of chitosan, it is possible to tailor the rate of drug release, thereby offering the opportunity for reduced frequency of dosing and improving patient compliance, whilst at the same time delivering a high respirable fraction.

2. Materials and methods

2.1. Materials

Terbutaline sulfate, low molecular weight (LMW: <190 kDa), medium molecular weight (MMW: 190–310 kDa) and high molecular weight (HMW: >310 kDa) chitosan, phosphate-buffered saline (PBS) tablets, α-lactose monohydrate and L-leucine were purchased from Sigma–Aldrich Chemicals (Poole, UK). HPLC grade methanol and ethanol were purchased from Fisher Scientific Ltd (Loughborough, UK).

2.2. Preparation of spray-dried powders

Formulations for spray-drying were prepared by the addition of an aqueous solution of terbutaline sulfate (model drug), leucine (aerosolisation enhancer [22]) and lactose (bulking agent) to a chitosan gel, prepared using LMW, MMW, HMW chitosan or combinations thereof.

LMW chitosan gel was prepared by mixing 4 g LMW chitosan in 100 mL glacial acetic acid aqueous solution (1.5% v/v) for 2 h. MMW chitosan gel was prepared by mixing 2.5 g MMW chitosan in 100 mL glacial acetic acid aqueous solution (0.5% v/v) for 2 h. HMW chitosan gel was prepared by mixing 2.7 g HMW chitosan in 100 mL glacial acetic acid aqueous solution (0.55% v/v) for 2 h. All preparations were allowed to stand overnight before use.

Sufficient chitosan gel to provide 1 g chitosan was measured and subsequently diluted with 30 mL ethanol to prepare LMW, LMW/MMW, MMW, MMW/HMW or HMW chitosan formulations. For example, to prepare the LMW chitosan formulation, 25 mL LMW chitosan gel (containing 1 g LMW chitosan) was mixed with 30 mL ethanol. An aqueous solution of 80 mg terbutaline sulfate, 720 mg leucine and 200 mg lactose was then combined with the chitosan ethanol mixture under homogenisation at 1600 rpm for 10 min to produce 100 mL of a 30% v/v aqueous ethanol solution [22] containing a total solid mass of 2% w/v (50% of which was chitosan). A control formulation (no chitosan) was prepared using 80 mg terbutaline sulfate, 720 mg leucine and 1.2 g lactose in 100 mL of 30% v/v aqueous ethanol solution.

The prepared formulations were subsequently spray-dried using a mini spray-dryer equipped with a high performance cyclone (Büchi B-290: Büchi Labortechnik AG, Switzerland) with a 0.7-mm two-fluid nozzle, using the following standard operating conditions: inlet temperature, 180 °C; spray flow rate, 600 L/h; pump setting, 10% (3.2 mL/min); aspirator setting, 85% (34 m^3/h). These conditions resulted in an outlet temperature of 84–92 °C. The resultant chitosan powders contained 4% w/w terbutaline, 36% w/w leucine, 50% w/w chitosan (LMW, LMW/MMW, MMW, MMW/HMW or HMW) and 10% w/w lactose. The control powder contained 4% w/w terbutaline, 36% w/w leucine and 60% w/w lactose.

2.3. Powder characterisation

2.3.1. Spray-drying yield and drug content

The yields of spray-dried powders were quantified as the percentage of anticipated yields. The terbutaline content of the powders was measured in triplicate, with analysis by high-performance liquid chromatography (HPLC), and expressed as the percentage of nominal load.

2.3.2. Scanning electron microscopy

Spray-dried powders were mounted onto separate, adhesive-coated, 12.5 mm diameter aluminium pin stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle-free compressed gas across each. The specimen stubs were sputter coated with a thin (approximately 10 nm) layer of gold in a Polaron SC500 coating unit at 10 mA for 2 min using an argon gas purge.

The specimens were examined using a Topcon SM-300 scanning electron microscope (SEM). The SEM was operated at high vacuum with an accelerating voltage of 5 kV and a specimen working distance of 12 mm. Secondary electron images were recorded digitally at a magnification 5000×.

2.3.3. Amorphous nature and water content

Determination of the degree of amorphous material and the water content in the spray-dried powders was performed using differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), respectively. DSC (Pyris Diamond DSC and Intracooler 2P: Perkin-Elmer, Wellesley, USA) was performed on 2 mg samples in aluminium pans using a nitrogen purge at 20 mL/min (range: ambient–300 °C, heating rate 20 °C/min). TGA (Pyris 1 TGA: Perkin-Elmer, Waltham, USA) was performed on 10 mg samples in platinum pans using a nitrogen purge at 20 mL/min (range: ambient – 360 °C, heating rate 50 °C/min). Measurements were performed in triplicate.

2.3.4. Particle size, powder density and primary aerodynamic diameter

The particle size of the spray-dried powders was measured by laser diffraction (HELOS particle size analyser incorporating VIBRO/RODOS dry dispersion system: Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, Germany). Approximately 100 mg of each powder was used to achieve the required obscuration of 5%, and each sample was measured in triplicate. The data obtained were expressed as the volume weighted mean particle size.

The poured density of the spray-dried powder was determined by pouring a known mass of powder (approximately 0.5 g) under gravity into a calibrated measuring cylinder and recording the volume occupied by the powder. The tapped density of the spray-dried powders was determined by tapped density measurements on the same samples using a tamping volumeter (Tapped Density Assessor: Copley Scientific Ltd., Nottingham, UK) until no further change in the powder volume was observed. Measurements were performed in triplicate. Carr's Index values for each spray-dried powder were derived from poured density and tapped density data, according to Eq. 1. The Carr's Index value gives an indication of powder flow; a value less than 25% indicates a fluid powder, whereas a value greater than 25% indicates a cohesive powder [41].

$$\text{Carr's Index } (\%) = \frac{100(\text{Tapped density} - \text{Poured density})}{\text{Tapped density}} \quad (1)$$

Theoretical estimates of particle primary aerodynamic diameter (d_{ae}) were derived from the particle sizing (d) and tapped density data (p), according to Eq. 2 [15].

$$d_{ae} = d\sqrt{\frac{\rho}{\rho_1}} \quad \text{Where } \rho_1 = 1 \text{ g cm}^{-3} \quad (2)$$

2.4. In vitro powder aerosolisation

The aerosolisation properties of the spray-dried powders were investigated using a Multi-Stage Liquid Impinger (MSLI, Copley Scientific Ltd., Nottingham, UK). HPLC mobile phase (20 mL) was introduced to Stages 1–4 of the MSLI, and a filter paper (Whatman GF-A) placed at Stage 5. The flow rate through the MSLI was adjusted to 60 L/min using an electronic digital flow meter (Model DFM2: Copley Scientific Ltd., Nottingham, UK). Aliquots of the spray-dried powders (3×25 mg) were loaded into size 2 hydroxypropyl methylcellulose (HPMC) capsules (Shionogi Qualicaps) and placed into a Spinhaler® dry powder inhaler (DPI), attached to the MSLI via a stainless steel USP throat. In contrast to conventional dry powder inhaler formulations that blend the fine particle drug powder with a coarse particle carrier powder such as lactose in order to improve the aerosolisation characteristics, the spray-dried powders generated in this study were not blended with a carrier powder, and were aerosolised simply as the spray-dried material. The capsule was pierced and the liberated powder drawn through the MSLI at a flow rate of 60 L/min for 2× 5 s aspirations using a pressure calibrator (Model TPK: Copley Scientific Ltd., Nottingham, UK). Under these conditions, the effective cut-off diameters are Stage 1: 6.8 μm, Stage 2: 3.1 μm, Stage 3: 1.7 μm, Stage 4: 0.26 μm, with Stage 5 as a terminal filter. Each deposition experiment was performed in triplicate.

The emitted dose (ED), defined as the percent of total loaded powder mass exiting the capsule, was determined gravimetrically. Subsequently, the mobile phase at each stage of the MSLI was removed for analysis. The inhaler, throat and filter were each washed with 20 mL mobile phase. HPLC was used to quantify the fraction of terbutaline recovered from the inhaler, throat, stages 1–4 and filter of the MSLI. The fine particle dose (FPD), defined as the mass of drug less than 5 μm, was calculated by interpolation from a plot of cumulative mass vs. effective cut-off

diameter of the respective stages. The fine particle fraction (FPF) was calculated as the ratio of FPD to total loaded dose, expressed as a percentage and corrected for actual terbutaline content in each powder. The mass median aerodynamic diameter (MMAD) of the powders was also derived, defined as the particle size at the 50% mark of a plot of cumulative fraction vs. effective cut-off diameter.

2.5. HPLC analysis of terbutaline

The mass of terbutaline deposited on each stage of the MSLI was determined using reverse-phase HPLC (Dionex AS50 autosampler with GP50 Gradient pump HPLC System: Dionex, UK) at room temperature using a 4.6×150 mm column (Phenomenex La Luna: Phenomenex, Torrance, USA) and 15 μl injection volume with UV detection at 276 nm. The mobile phase (1 mL/min) consisted of 23% v/v aqueous methanol, with terbutaline eluting with a retention time of 2.5 min.

2.6. In vitro dissolution testing

Dissolution testing was performed on 200 mg spray-dried powder using the Modified USP II dissolution apparatus (Hanson Research SR6 Dissolution Test Station: Hanson Research Ltd, Chatsworth, USA; Caleva SG6 and 65G Dissolution Apparatus: Caleva Ltd, Dorset, UK; or Sotax A7 Dissolution Apparatus: Sotax Ltd, London, UK) with 2 cm diameter stainless steel wire baskets (Copley Scientific Ltd, Nottingham, UK), rotating at 50 rpm in 1000 mL PBS (37 °C, pH 6.8). Samples (3 mL) were withdrawn for analysis at specified time points, and assessed for terbutaline content by UV spectroscopy (Jenway 6305 UV–vis spectrophotometer: Jenway, Essex, UK) at 276 nm; the sample was returned to the bath immediately after analysis. Each dissolution experiment was performed in triplicate.

2.7. Statistical analysis

The drug loading, emitted dose, FPD and FPF of the chitosan spray-dried powders were statistically compared to those of the control spray-dried powder using one-way analysis of variance with Dunnett multiple comparison test. Where appropriate, the aerosolisation properties of the chitosan powders were compared against each other using one-way analysis of variance with Tukey–Kramer multiple comparisons test. The significance level was 0.05.

3. Results and discussion

3.1. Spray-dried powder characteristics

Use of the high performance cyclone resulted in the collection of high yields of the spray-dried powders (range: 59–79% of anticipated amount; Table 1). Analysis of the terbutaline content of the spray-dried powders indicated that the drug loading ranged from 94% to 117% of the anticipated amount (Table 1); statistical analysis indicated that the drug content of each powder was similar (one-way ANOVA/Dunnett and one-way ANOVA/Tukey–Kramer: not significant).

Scanning electron microscopy was used to visualise the particle diameter, structural and surface morphology of the spray-dried powders (Fig. 1). Interestingly, the micrograph of the control powder did not show spherical particles (Fig. 1a); rather, the particles appear to have undergone fusion and partial recrystallisation. This may have occurred either during storage or possibly at some stage during SEM processing. In contrast, the micrographs of the chitosan-modified spray-dried powders (Fig. 1b–f) indicated that the powders comprised regular spherical particles with a diameter of less than 10 μm. The particles appear to have an undulating surface, and this is particularly apparent for the HMW chitosan powder (Fig. 1f). Most striking about the micrographs of the chitosan-modified powders is the appearance of whisker-like material clinging to the surface of the particles. Asada and co-workers [36] noted that similar asperities developed on the surface of theophylline–chitosan spray-dried particles over time, and attributed this phenomenon to theophylline undergoing capillary condensation. It is unclear whether this phenomenon is occurring with the terbutaline in our chitosan-based spray-dried powders, or whether the surface-deposited material is excess leucine or chitosan which precipitated from the spray-drying formulation during spray-drying.

The SEM images suggested that the powders formed were amorphous in nature; this was as expected, as powders generated through spray-drying are known to be predominately amorphous in nature [42]. This observation was confirmed by differential scanning calorimetry. Given the amorphous nature of the powders, all samples were stored at room temperature in a desiccator immediately after spray-drying to limit crystallisation of the samples between powder production and aerosolisation testing. Thermogravimetric analysis of the spray-dried powders indicated that the moisture content of the powders ranged from 1.2% to 5.9% w/w (Table 1). It was noticed that the chitosan formulations had a higher water content than the control powder; this could be due to water being retained in the chitosan matrix during spray-drying. Even so, these values are in line with other studies that indicate moisture content of spray-dried powders to be up to 7.5% w/w [43,44].

Laser diffraction data are presented in Table 1, with a representative size distribution displayed in Fig. 2. The mean particle size of each of the spray-dried powders was less than 10 μm, with the control powder and the MMW chitosan powder exhibiting the smallest and largest particle size, respectively (4.3 and 9.9 μm, respectively). It is interesting to note the variation in particle size, given that all formulations contained the same mass of solid (2% w/v) and were spray-dried using standard conditions. It is likely

Table 1
Physical characterisation of spray-dried powders (values are means ± SD, $n = 3$)

Powder	SD yield (%)	Drug loading (%)	Water content (%)	Particle size (μm)	Tapped density (g/cm^{-3})	Carr's Index[a]		d_{ae} (μm)
						(%)	Flowability	
Control	73	116.9 ± 11.2	1.22 ± 0.01	4.31 ± 0.32	0.19 ± 0.02	22.2	Poor, fluid	1.87 ± 0.17
LMW	79	107.8 ± 17.4	3.17 ± 0.16	5.65 ± 0.18	0.16 ± 0.00	30.9	Poor, cohesive	2.23 ± 0.08
LMW/MMW	63	93.8 ± 1.4	4.14 ± 0.04	6.01 ± 0.63	0.19 ± 0.01	30.7	Poor, cohesive	2.61 ± 0.35
MMW	56	103.1 ± 7.2	5.63 ± 0.20	9.93 ± 0.74	0.12 ± 0.01	26.3	Poor, cohesive	3.40 ± 0.32
MMW/HMW	62	99.6 ± 8.3	5.86 ± 0.32	6.41 ± 0.40	0.19 ± 0.01	17.5	Good	2.76 ± 0.15
HMW	59	101.3 ± 12.0	2.55 ± 0.17	7.88 ± 3.08	0.13 ± 0.01	22.6	Poor, fluid	2.85 ± 1.12

[a] Carr's Index flowability: 5–12%, excellent; 12–18%, good; 18–21%, fair; 21–25%, poor, fluid; 25–32%, poor, cohesive; 32–38%, very poor; >40%, extremely poor (from [41]).

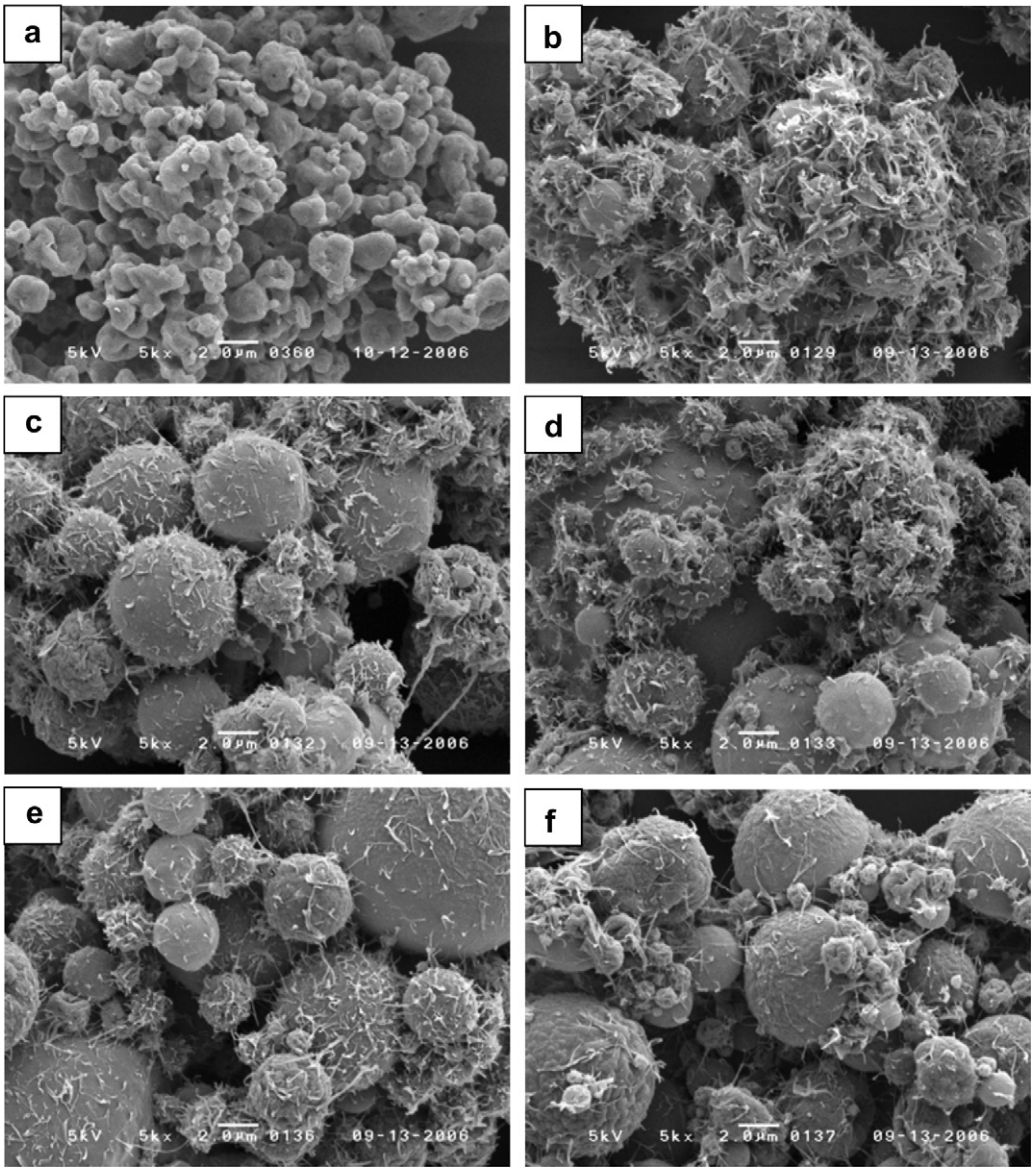

Fig. 1. Representative scanning electron micrographs of control and chitosan-modified spray-dried powders. (a) control powder, (b) LMW chitosan powder, (c) LMW/MMW chitosan powder, (d) MMW chitosan powder, (e) MMW/HMW chitosan powder, (f) HMW chitosan powder. Bar = 2 μm.

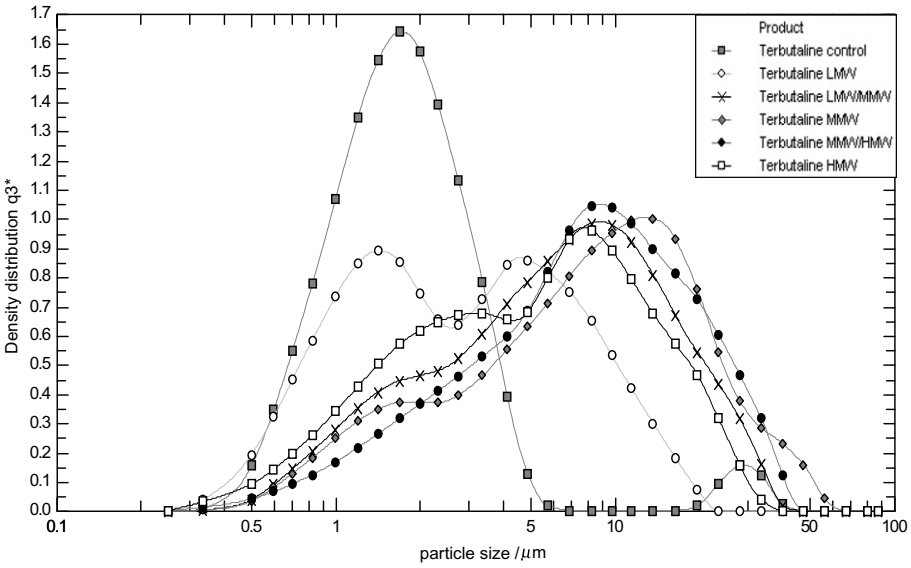

Fig. 2. Particle size distribution of control and chitosan-modified spray-dried powders.

that the inclusion of chitosan gel in the formulation increased the viscosity of the liquid being spray-dried; this could result in a larger droplet at the spray-drying nozzle, which would spray-dry to give a larger particle, as previously suggested by other researchers (e.g. [45]). In addition, comparison of the SEM images and the laser diffraction data suggests that during particle sizing, the chitosan-modified powders did not behave as individual particles, rather as particle aggregates (as evidenced by the wide distribution). The tapped density of the spray-dried powders was similar for all powders (range: 0.12–0.19 g cm^{-3}; Table 1), and is in line with previous investigations in these laboratories (e.g. [22]). The laser diffraction and tapped density data were used to calculate the theoretical primary aerodynamic diameter (d_{ae}). As shown in Table 1, the d_{ae} value of all spray-dried powders was between 1.9 and 3.4 μm, suggesting that the powders were of a suitable aerodynamic size for pulmonary delivery.

Carr's Index may be used as an indication of powder flow properties; a value less than 25% indicates a fluid flowing powder, whereas a value greater than 25% indicates cohesive powder characteristics [41]. The Carr's Index values of these spray-dried powders ranged from 17.5% (MMW/HMW chitosan powder: good flowability) to 30.9% (LMW chitosan powder: poor, cohesive flowability). Interestingly, the control powder had a Carr's Index of 22.2%, indicating poor, fluid flowability; this suggests that some of the chitosan formulations had better flowability than the control powder, whereas other formulations had poorer flowability.

3.2. In vitro powder aerosolisation

The ED, FPD, FPF and MMAD of the spray-dried powders are displayed in Table 2. All powders showed high dispersibility, with at least 90% of the capsule contents being emitted during aerosolisation testing. Statistical analysis indicated that there was no difference in the ED values of the different powders (one-way ANOVA/Dunnett and one-way ANOVA/Tukey–Kramer: not significant). This high dispersibility is clearly a reflection of the aerodynamic properties of the spray-dried powders, arising from the inclusion of leucine as a dispersibility enhancer [20,22].

The MSLI deposition pattern of the spray-dried powders is shown in Fig. 3. Of particular interest is the very low deposition in the inhaler and throat regions, again supporting the highly dispersible nature of these powders; following inhalation, minimal deposition in the oropharyngeal region would be expected, thereby reducing the potential for local side effects. The control powder exhibited

Table 2
Aerosolisation characteristics of spray-dried powders (values are means ± SD, $n = 3$)

Powder	Emitted dose (%)	Fine particle dose (μg)	Fine particle fraction (%)	MMAD (μm)
Control	95.2 ± 2.2	2804.8 ± 207.2	74.9 ± 10.4	1.66 ± 0.18
LMW	94.2 ± 4.6	2572.6 ± 85.9	82.0 ± 3.1	2.00 ± 0.11
LMW/MMW	96.9 ± 3.8	2091.8 ± 148.2**	73.4 ± 3.2	1.76 ± 0.08
MMW	95.9 ± 1.2	2317.2 ± 212.3*	68.5 ± 7.0	1.85 ± 0.52
MMW/HMW	96.4 ± 3.0	1940.3 ± 74.9**	65.7 ± 3.0*	1.95 ± 0.47
HMW	97.3 ± 0.6	1908.3 ± 335.5**	55.8 ± 1.5**	2.30 ± 0.70

Statistical difference (one-way ANOVA/Dunnett) from control.
* $p < 0.05$.
** $p < 0.01$.

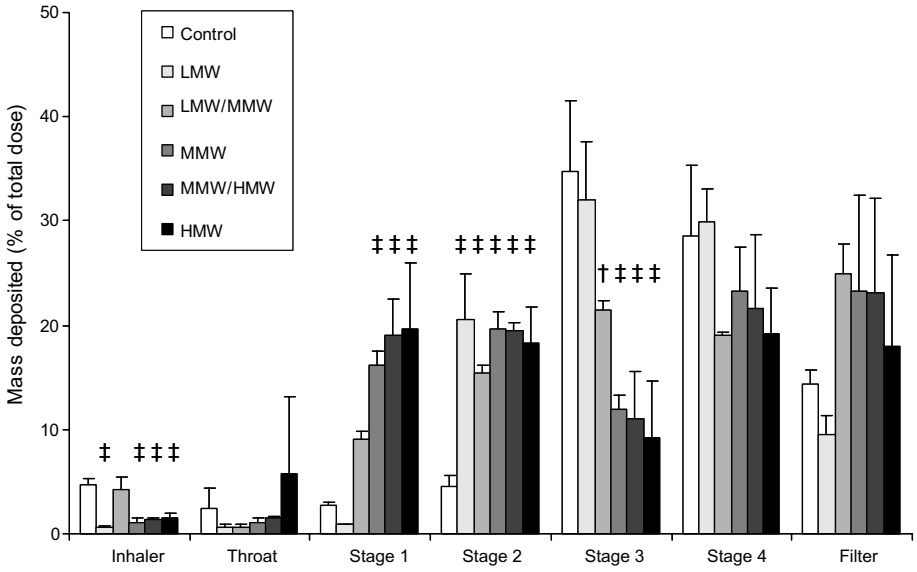

Fig. 3. Multi-stage liquid impinger deposition profile for control and chitosan-modified spray-dried powders, expressed as the percentage of total loaded dose. Values are means ± SD, $n = 3$. Statistical difference (one-way ANOVA/Dunnett) from control powder at that stage of the MSLI: $^{\dagger}p < 0.05$, $^{\ddagger}p < 0.01$.

minimal deposition on Stage 1 and Stage 2 of the MSLI, with over 75% of the powder deposited on Stages 3-Filter. Inclusion of chitosan in the formulation significantly increased the deposition of powder on the earlier stages of the MSLI (one-way ANOVA/Dunnett: $p < 0.01$), although substantial deposition was still evident at the lower stages. For example, the mass deposited on Stages 3-Filter for the LMW, MMW and HMW chitosan powders was 71%, 58% and 46%, respectively. All powders would therefore be expected to perform well during inhalation, and to deliver a large proportion of the dose to the central regions of the lung. Indeed, the high proportion of powder reaching as far as Stage 4 and Filter suggests that these powders may even be of use for the delivery of drugs for peripheral lung activity or systemic uptake.

The nominal dose in each aerosolisation test was 3 mg terbutaline sulfate (25 mg powder per capsule, 4% w/w drug loading, three capsules per test). The control powder exhibited the highest FPD, whereas the HMW chitosan powder exhibited the lowest FPD (2.8 and 1.9 mg terbutaline sulfate, respectively; Table 2). The FPD of the control powder was statistically higher than that of all chitosan powders except the LMW chitosan powder (one-way ANOVA/Dunnett: $p < 0.05$). The FPF of each spray-dried powder, corrected for actual drug content, ranged between 56% and 82% of total capsule contents. The FPF of the MMW/HMW and the HMW chitosan powders (66% and 56%, respectively) were significantly (one-way ANOVA/Dunnett: $p < 0.05$) lower than that of the control powder (75%), whereas the FPF of the LMW, LMW/MMW and MMW chitosan powders were statistically similar to the control powder. In addition, the FPF of the LMW chitosan powder was statistically higher than that of the MMW, MMW/HMW and HMW chitosan powders (one-way ANOVA/Tukey–Kramer: $p < 0.05$).

The MMAD of the spray-dried powders (range: 1.7–2.3 μm) was in line with the theoretical estimates of d_{ae}. Other investigators have noted that aerosolisation of spray-dried powders can result in MMAD values much greater than the theoretical estimates of aerodynamic diameter derived from powder density and physical diameter, and concluded that this is due to the particles remaining as aggregates during aerosolisation (e.g. [15]). In contrast, the similarity of the d_{ae} and MMAD value for our spray-dried powders suggests that they did in fact behave as individual particles rather than particle aggregates during aerosolisation. This may be due to the high proportion of leucine in these powders. Leucine is a particularly hydrophobic amino acid [46,47], and its surfactant-like properties may result in the capacity for leucine to migrate to the droplet surface during the rapid drying phase in spray-drying, and hence influence the surface characteristics of the resultant particle [22,48,49], resulting in highly dispersible particles that display optimal aerosolisation properties. In addition, researchers have shown that chitosan can enhance the dispersibility of spray-dried powders [50], and it is feasible the chitosan is not only acting as a drug release modifier, but also modifying the surface of the powder particles, decreasing interparticulate cohesion and thereby improving powder dispersibility.

As noted above, the FPF of the MMW/HMW and HMW chitosan-modified powders was statistically lower than that of the unmodified and the LMW powders. In addition, a trend suggesting that increasing the molecular weight of the chitosan decreased the FPF was observed (Fig. 4). It is feasible that inclusion of chitosan of increasing polymer chain length increased the viscosity of the spray-drying formulation, thereby influencing the ability of leucine to migrate to the surface of the droplet during the spray-drying process. This could therefore have a detri-

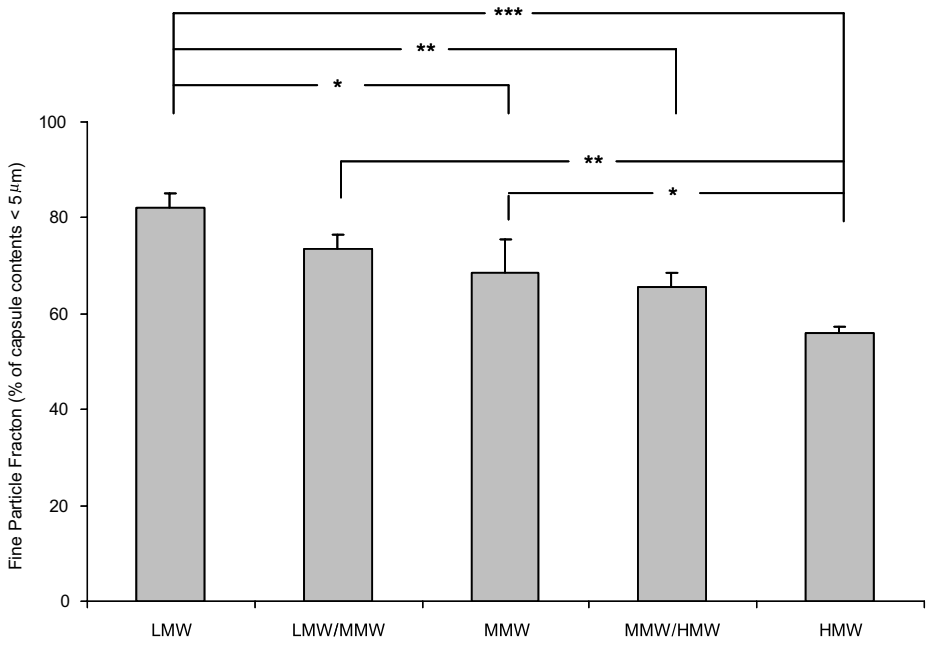

Fig. 4. Fine particle fraction (FPF) of chitosan-modified spray-dried powders. Values are means ± SD, $n = 3$. Statistical difference (one-way ANOVA/Tukey–Kramer) between powders: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

mental effect on the surface properties of the resultant spray-dried particles, resulting in increased interparticulate cohesion and decreased deaggregation of the particles during aerosolisation, resulting in a larger MMAD and a lower FPF. However, it should be noted that although the HMW chitosan powder exhibited the lowest FPF of the powders tested in this study, an FPF of 56% is still higher than those reported by other researchers for less complex powders (e.g. [51–53]. All powders tested in the present study would be expected to deliver a high proportion of the total capsule contents to the pulmonary region following inhalation, with limited deposition in the device, oropharyngeal region or upper respiratory tract.

3.3. In vitro dissolution

Although several in vitro models exist for the prediction of respirable fraction and site of deposition in the lung following pulmonary administration (e.g. MSLI, Andersen Cascade Impactor, Next Generation Impactor, Twin Stage Impinger), there is no readily available in vitro model to predict the rate and extent of drug dissolution in the lung following inhalation. Literature references to dissolution models for inhalable powders are rare [25]; this is somewhat surprising, given that the rate of dissolution of a drug once inhaled is critically linked to onset and duration of therapeutic activity. In the absence of a more appropriate model, standard in vitro powder dissolution testing was used to provide a comparison between the dissolution profile of the control spray-dried powder and the chitosan-modified spray-dried powders.

As expected, the control powder underwent very rapid dissolution, with 100% terbutaline released after approximately 5 min (Fig. 5). The chitosan powders exhibited delayed release characteristics; increasing the MW of the chitosan was associated with a more sustained release profile. For example, the LMW chitosan powder released 100% terbutaline after 30 min, whereas 2 h dissolution time was necessary for the HMW chitosan powder to release its entire drug load. The MMW chitosan powder displayed an intermediate release profile, with 100% drug release after 60 min. Interestingly, the mixed chitosan powders (LMW/MMW and MMW/HMW) did not perform precisely as expected. It was anticipated that the LMW/MMW chitosan powder would exhibit a release profile somewhere between that of the LMW and MMW chitosan powders; the LMW/MMW chitosan powder actually demonstrated a release profile akin to that of the LMW chitosan powder. Likewise, the release profile of the MMW/HMW chitosan powder was not as anticipated, and was more analogous to that of the HMW chitosan powder.

The term "chitosan" actually refers to a series of chitosan polymers with different molecular weights [26], and the batches of chitosan used in these studies were classified by the supplier as "low", "medium" and "high" molecular weight. Although these terms are not particularly precise, they do provide information on the relative length of the chitosan polymer chain. Previous investigators have suggested that when microspheres containing hydrophilic polymers such as chitosan are immersed in water, diffusion of the drug through a gel diffusion layer produced through polymer swelling results in a sustained drug release effect. Increasing the amount of chitosan in the microsphere increases the thickness of this diffusion layer, resulting in greater retention of drug release [31,54]. In our studies, we maintained a constant amount of chitosan in the

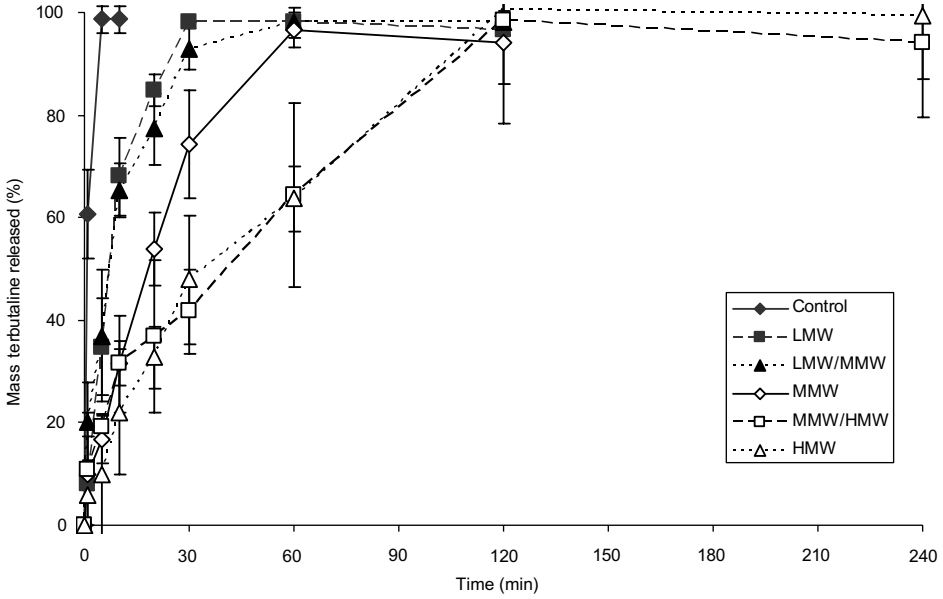

Fig. 5. Terbutaline release from control and chitosan-modified spray-dried powders.

spray-drying formulation, and the resultant spray-dried microspheres contained 50% w/w chitosan; rather than change the proportion of chitosan in these powders, we modified the release characteristics through the use of low, medium and high molecular weight chitosan. During dissolution of the chitosan powders, the formation of a thick gel was observed, which expanded slowly over time. This suggests that dissolution occurred through a matrix-like release system resulting from the mechanical interlocking of the long polymer chains, with the potential for hydrogen bond formation between the hydroxyl groups on the chitosan and terbutaline molecules retarding drug release. We hypothesised that the longer polymer chains present in HMW chitosan would give rise to greater mechanical interlocking and hydrogen bond formation and thereby provide the most prolonged drug release profile; the dissolution results appear to support this, as the HMW chitosan powder demonstrated a substantially longer dissolution profile compared to the LMW and MMW chitosan powders.

To explore further the difference between the LMW, MMW and HMW chitosan powders, the percent dose released was plotted as a function of the square root of time (Fig. 6). In each case, a linear relationship was observed; the correlation coefficients (r^2 values), rate of drug release (obtained from the gradient of the line), the lag time (calculated from the intercept on the x-axis), and the time taken for 50% drug release are presented in Table 3. Similar correlation to square-root-time kinetics for chitosan formulations has been observed by Zambito and Di Colo [55], who suggested that the rate of drug release from chitosan matrices is determined by the time taken

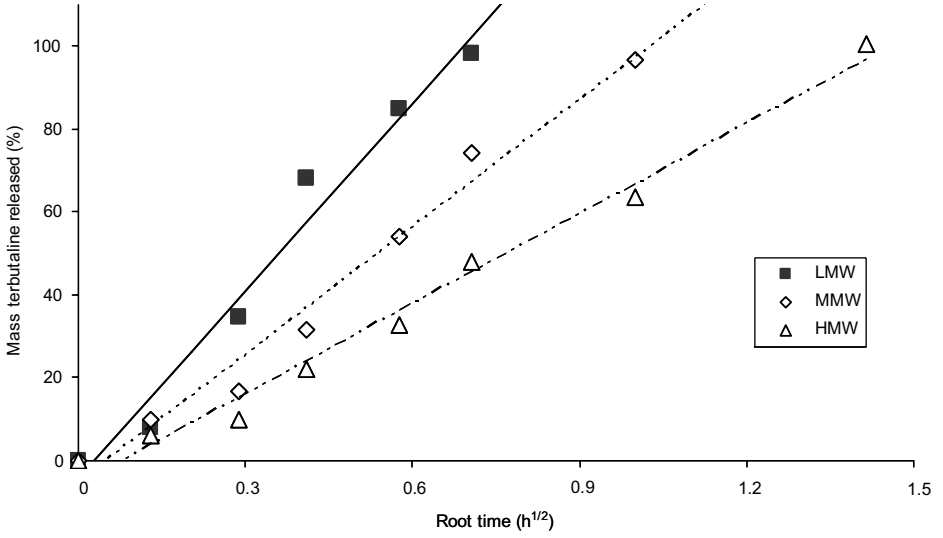

Fig. 6. Square-root time analysis of terbutaline release from LMW, MMW and HMW chitosan-modified spray-dried powders.

Table 3
Square-root time analysis of terbutaline release from LMW, MMW and HMW chitosan-modified spray-dried powders

Powder	Correlation coefficient (r^2)	Rate of release ($h^{-0.5}$)	Lag time (s)	$t_{50\%}$ (min)
LMW	0.9729	150.63	4.51	7.70
MMW	0.9787	102.40	12.13	17.23
HMW	0.9863	72.22	24.88	35.20

to hydrate the chitosan (the lag time) and the dissolution and diffusion of the drug through an aqueous path created in polymer following hydration. In our studies, a clear trend can be seen between the molecular weight of chitosan, the lag time and the rate of drug release, with the HMW chitosan powder demonstrating a longer lag time and lower rate of drug release than the MMW and LMW chitosan powders.

Naturally, in vitro dissolution tests of this nature do not take into consideration the environment that would be encountered following inhalation, such as the relatively low quantity of lung secretions, the large surface area, the lung's clearance mechanisms such as the mucociliary escalator and the mucus layer and the presence of lung surfactant. Nevertheless, these studies do give comparative information about the rate of drug release from different formulations, and whilst the dissolution times observed in these studies would not be predicted in vivo, one would still anticipate a faster release profile from the LMW chitosan powder compared to the HMW powder. These studies therefore demonstrate the potential to tailor the drug release profile by the appropriate selection of chitosan MW.

4. Conclusions

These investigations demonstrate that it is possible to generate highly respirable powders that exhibit a sustained drug release profile. Increasing the molecular weight of the chitosan incorporated during spray-drying reduces the fine particle fraction of the powder, but substantially increases the time taken for drug release. These powders would be predicted to deposit predominately in the central and peripheral regions of the lung following inhalation, with minimal oropharyngeal deposition, thereby maximising the dose delivered to the lung and reducing the incidence of oropharyngeal side-effects. Once deposited in the lung, these powders would be anticipated to display a delayed rather than instantaneous drug release profile, offering the opportunity to reduce dosing frequency.

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