International Journal of Pharmaceutics 372 (2009) 97–104

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Sustained delivery by leucine-modified chitosan spray-dried respirable powders

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

[a] School of Life and Health Sciences, Aston University, Birmingham, UK
[b] School of Pharmacy, University of Hertfordshire, Hatfield, Herts., UK
[c] Pfizer Global Research and Development, Kent, UK

ARTICLE INFO

Article history:
Received 17 October 2008
Received in revised form 13 January 2009
Accepted 15 January 2009
Available online 24 January 2009

Keywords:
Inhalation
Leucine
Modified release
Spray drying
Thermal efficiency

ABSTRACT

The controlled co-delivery of multiple agents to the lung offers potential benefits to patients. This study investigated the preparation and characterisation of highly respirable spray-dried powders displaying the sustained release of two chemically distinct therapeutic agents. Spray-dried powders were produced from 30% (v/v) aqueous ethanol formulations that contained hydrophilic (terbutaline sulphate) and hydrophobic (beclometasone dipropionate) model drugs, chitosan (as a drug release modifier) and leucine (aerosolisation enhancer). The influence of chitosan molecular weight on spray-drying thermal efficiency, aerosol performance and drug release profile was investigated. Resultant powders were physically characterised: with *in vitro* aerosolisation performance and drug release profile investigated by the Multi-Stage Liquid Impinger and modified USP II dissolution apparatus, respectively. It was found that increased chitosan molecular weight gave increased spray-drying thermal efficiency. The powders generated were of a suitable size for inhalation—with emitted doses over 90% and fine particle fractions up to 72% of the loaded dose. Sustained drug release profiles were observed in dissolution tests for both agents: increased chitosan molecular weight associated with increased duration of drug release. The controlled co-delivery of hydrophilic and hydrophobic entities underlines the capability of spray drying to produce respirable particles with sustained release for delivery to the lung.

1. Introduction

The advent of multiple treatments for chronic obstructive respiratory disease has had a positive effect in the management of life threatening pulmonary disease. However, the management of lung disease pharmacotherapy is heavily dependent up on patient compliance and concordance with regimens of three or more devices (Holgate and Polosa, 2008, 2006). In addition, the vast majority of patients on medication for obstructive lung disease experience breakthrough symptoms and acute exacerbations of the disease over an extended time period; such breakthroughs are often associated with poor medication compliance or disease management (Holgate and Polosa, 2006; Barnes, 2006).

The introduction of dry powder 'combination' inhalers to the global market, incorporating a long-acting β2 agonist with a long-acting corticosteroid, has aided obstructive lung disease management (Holgate and Polosa, 2008). However, the addition of a short-acting β2 agonist to the long-acting β2 agonist and the corticosteroid contained in the 'polyhalers' is recommended to treat breakthrough symptoms (Holgate and Polosa, 2006; Balanag et al., 2006; Theophilus et al., 2006). The delivery of a highly efficacious short-acting β2 agonist with a corticosteroid to the bronchi, as part of a sustained release formulation, could theoretically simplify dose regimens by reduced frequency of administration and elimination of a secondary reliever inhaler, and so improve asthma management (Cazzola and Matera, 2008).

Traditional polyhaler dry powder inhalers (DPIs) use two micronised drug powders (of <5 μm in size) blended with a coarse carrier particle population (60–90 μm) (Taki et al., 2006). A problem when using the traditional polyhaler formulation is the inability to develop sustained release characteristics without an additional step to the micronisation of the components and blending. Therefore long-acting agents such as the long-acting β2 agonist salmeterol are required to achieve the recommended twice day dosing of the current crop of marketed polyhalers (British National Formulary, 2007). With the necessary addition of a short-acting β2 agonist to the regimen the convenience of the polyhaler is lost. This research aims to produce a long-acting therapy for asthma using the sustained release of short-acting agents from polymer encapsulated spray-dried formulations.

Spray drying also offers the option of a one-step continuous process away from the two-step micronisation and blending phases of formulation of the traditional polyhalers. As previously documented, spray drying has been utilised as an alternative method

* Corresponding author at: Medicines Research, School of Pharmacy, University of Hertfordshire, College Lane, Hatfield AL10 9AB, UK. Tel.: +44 1707 281334; fax: +44 1707 284506.
E-mail address: T.Learoyd@herts.ac.uk (T.P. Learoyd).

0378-5173/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2009.01.017

to produce dry particles within a respirable size range (Learoyd et al., 2007, 2006a,b). This novel study demonstrates how spray-dried chitosan (CT) formulations containing the dispersibility enhancer leucine (LEU) can generate highly respirable powders, which give the simultaneous latent delivery of both a hydrophilic (terbutaline sulphate: TS) and a hydrophobic (beclometasone dipropionate: BDP) marker (Learoyd et al., 2008a).

There are many advantages to developing sustained release formulations in pulmonary drug delivery, including reduced dosing frequency, improved patient compliance and reduction of side-effects (Hardy and Chadwick, 2000). When the use of a polymer is employed as a rate-limiting factor to achieve sustained drug dissolution a partially water insoluble vector is usually indicated, the elected polymer in this study was the crustacean derived polysaccharide CT (Kim et al., 2006; Illum, 1998).

Barriers associated with the delivery of formulations to the lungs include the geometries and areas of turbulence within the human lung, which require a particulate to be of an aerodynamic size of typically less than 5 μm. A further consideration when delivering particulates for controlled delivery is the mucocilliary clearance mechanism of the lung which can move particles from the target bronchioles to the throat at a rate of ≤12 mm/min (Hickey and Thompson, 1992). A suitable mucoadhesive component to any formulation must therefore be considered. Further consideration must be taken as to which polymer should be incorporated because foreign high molecular weight additives can be toxic on accumulation in the lung. Bronchiole mucus plugging may also be a problem when the formulation is gel forming.

CT, an amino-polysaccharide produced by the deacetylation of the naturally occurring polymer chitin, is insoluble in water until acidified into a gel structure of interlocking chains; a promising excipient with a wide range of applications, CT can be used to promote sustained release in spray-dried preparations (Illum, 1998). Given that CT not only acts as a drug release modifier, but also has mucoadhesive properties (Martinac et al., 2005; Harikarnpakdee et al., 2006; Dang and Leong, 2006; Sigurdsson et al., 2006), 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 CT formulations, only a handful of researchers have investigated the viability of CT-modified spray-dried powders for pulmonary drug delivery (Huang et al., 2003; Asada et al., 2004; Grenha et al., 2005; Corrigan et al., 2006), with none apparently having considered the incorporation of dispersibility enhancers such as leucine to improve powder aerosolisation character.

2. Materials and methods

2.1. Materials

TS; BDP; crude low molecular weight (LMW: <190 kDa), medium molecular weight (MMW: 190–310 kDa) and high molecular weight (HMW: >310 kDa); ≥75% deacetylated CT; phosphate buffered saline (PBS) tablets; reagent grade α-lactose monohydrate and pharmaceutical grade 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

LMW, MMW and HMW CT gels were prepared as outlined previously (Learoyd et al., 2008a). Sufficient CT 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 CT formulations. An aqueous solution of 80 mg TS and 80 mg BDP, 720 mg LEU and 120 mg lactose was then combined with the CT ethanol mixture under homogenisation at 1600 rpm for 10 min to produce 100 mL of a 30% (v/v) aqueous ethanol solution which contained a total solid mass of 2% (w/v) (50% (w/w) of which was CT). Five spray-dried formulations were prepared ($n = 3$): 30% (v/v) aqueous ethanol solutions of TS, BDP, LEU (aerosolisation enhancer), lactose (bulking agent) and CT (prepared using LMW, MMW, HMW CT gels or combinations thereof) all comprising of 2% (w/v) (Learoyd et al., 2008a). An additional preparation of a control formulation of 4% (w/w) TS, 4% (w/w) BDP, 36% (w/w) LEU and 56% (w/w) lactose was prepared from a 2% (w/w) aqueous ethanol (30%, w/v) solution (Table 1).

Table 1
Chemical composition of control and 50% (w/w) chitosan (CT) spray-dried powders.

Formulation	% (w/w) chemical position						
	TS	BDP	Chitosan			Leucine	Lactose
			LMW	MMW	HMW		
Control	4	4	0	0	0	36	56
LMW	4	4	50	0	0	36	6
LMW/MMW	4	4	25	25	0	36	6
MMW	4	4	0	50	0	36	6
MMW/HMW	4	4	0	25	25	36	6
HMW	4	4	0	0	50	36	6

TS: terbutaline sulphate; BDP: beclometasone dipropionate; LMW: low molecular weight; MMW: medium molecular weight; HMW: high molecular weight.

The prepared formulations were subsequently spray-dried using a Büchi B-290 mini spray-dryer, equipped with a high performance cyclone (Büchi Labortechnik AG, Switzerland) and a 0.7-mm two-fluid nozzle atomiser, using the following 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). The resulting actual inlet (T_1), outlet (T_0) and environmental temperatures (T_2) were observed and the thermal efficiency (ω) calculated according to Eq. (1) (Masters, 1991).

$$\omega = \left(\frac{T_1 - T_2}{T_1 - T_0}\right) 100 \quad (1)$$

2.3. Powder characterisation

2.3.1. Spray drying yield and drug content

The yields of spray-dried powders were quantified as a percentage mass of the anticipated 2 g total powder yield. The TS and BDP content of each prepared powder was measured in triplicate, with analysis by HPLC, and expressed as the percentage of nominal load. Briefly, 20 mg samples of each formulation were diluted in 20 mL 23% aqueous HPLC grade methanol (elected mobile phase) and stored at room temperature for ten days in complete darkness. Samples were then assessed by the HPLC method detailed in Section 2.3.6. An additional test of the solubility of BDP in 23% (v/v) aqueous methanol showed saturation beyond 4 mg/mL.

2.3.2. Scanning electron microscopy (SEM)

Spray-dried powders were mounted onto separate, adhesive-coated, 12.5 mm diameter aluminium pin stubs with excess powder 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 SEM. The SEM was operated at high vacuum with an accelerating voltage of 5 kV and a worked specimen 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 water content in the spray-dried powders was performed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA), respectively. Linear DSC (Pyris Diamond DSC; Intracooler 2P: PerkinElmer, Wellesley, USA) was performed on 2 mg samples, mechanically sealed in aluminium pans, using a nitrogen purge at 20 mL/min (range: ambient −300 °C, heating rate 20 °C/min). TGA (Pyris 1 TGA: PerkinElmer, 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 analyzer incorporating VIBRO/RODOS dry dispersion system: Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, Germany). HELOS fitted with optical lens for 0.1–875 μm size range and 2000/s autofocus image capture, with the data processed by Windox 5.0 software. 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. (2). 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.

$$\text{Carr's index}\,(\%) = \frac{(\text{tapped density} - \text{poured density})}{\text{tapped density}} \times 100 \qquad (2)$$

Theoretical estimates of particle primary aerodynamic diameter (d_{ae}) were derived from the particle sizing (d) and tapped density data (p), according to Eq. (3).

$$d_{ae} = d\sqrt{\frac{p}{p_1}} \qquad (3)$$

2.3.5. In vitro powder aerosolisation

The aerosolisation properties of the spray-dried powders were investigated by a Multi-Stage Liquid Impinger (MSLI, Copley Scientific Ltd., Nottingham, UK). HPLC mobile phase (23%, v/v aqueous methanol, 20 mL (Learoyd et al., 2008a,b)) 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® DPI, attached to the MSLI via a stainless steel USP throat. 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 by a pressure calibrator (Model TPK: Copley Scientific Ltd., Nottingham, UK). Under these conditions, the effective cut-off diameters were: Stage 1: 13.0 μm, Stage 2: 6.8 μm, Stage 3: 3.1 μm, Stage 4: 1.7 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 TS and BDP 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 calculated from the determined drug contents of 20 mg samples (Section 2.3.1), expressed as a percentage of TS and BDP 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.

2.3.6. HPLC analysis of TS and BDP

The mass of TS and BDP 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 by a 4.6 mm × 150 mm regular packed silicone column (Phenomenex La Luna: Phenomenex, Torrance, USA) and 15 μL injection volume, with UV detection at 276 nm for TS and 250 nm for BDP. The mobile phase (1 mL/min) consisted of 23% (v/v) aqueous methanol, with a slight solvent front appearing at a 0.5-min retention time, TS eluting at a retention time of 2.5 min and BDP at 5 min during a single run as recorded in previous methods (Learoyd et al., 2008a,b). Limit of detection (LOD) for TS 0.69 μg/mL, limit of quantification (LOQ) 0.93 μg; BDP: LOD 0.98 μg/mL and LOQ 1.23 μg/mL.

2.3.7. In vitro dissolution testing

Dissolution was performed as previously outlined (Learoyd et al., 2008a,b). Briefly, testing was performed on 100 mg (4 mg TS, 4 mg BDP) spray-dried powder using 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); with the modification of samples being rotated at 50 rpm in 1000 mL PBS (37 °C, pH 6.8) (Shaw et al., 2002). Samples (3 mL) were withdrawn for analysis at specified time points, and assessed for TS and BDP content by UV spectroscopy (Jenway 6305 UV–vis spectrophotometer: Jenway, Essex, UK) at 276 nm and 250 nm, respectively. The sample was returned to the bath immediately after analysis. Each dissolution experiment was performed in triplicate. Sink conditions were observed for TS (solubility 0.9%, saline (pH 6.8): 7.789 mg/mL) (Panigrahi et al., 2005). However, BDP approached maximum solubility in the dissolution media (<0.01 mg/mL (Reid et al., 2008)) and encroached non-sink conditions c.25% cumulative release, with the solubility of leucine recognized as 0.02 mg/mL and the solubility of lactose determined as 216 mg/mL (Black and Mould, 1991; Machado et al., 2000). The appropriateness of the dissolution assay was determined by the BDP content of 100 mg spray-dried samples (stored in 1000 mL, 37 °C PBS pH 6.8, $n = 3$) for ten days. Spray-dried samples recording calculated pre-hydration depths of 1.7–2.5 mm and a surface area of 7.35–7.87 mm^2. The samples where calibrated against a series of 0.5 μg/mL, 1 μg/mL, 2 μg/mL, 3 μg/mL, 4 μg/mL, 5 μg/mL crystalline dilutions; the BDP release from the chitosan preparations recording 91.9–113.2% of the theoretical BDP content of 4 mg.

2.3.8. Statistical analysis

The drug loading, emitted dose, FPD and FPF of the CT spray-dried powders were statistically compared to those of the control

spray-dried powder using one-way analysis of variance (ANOVA) with the Dunnett multiple comparison test. Where appropriate, the aerosolisation properties of the CT powders were compared against each other using one-way analysis of variance with Tukey–Kramer multiple comparisons test unless stated. The significance level was 0.05.

3. Results and discussion

3.1. Spray-dried powder characteristics

The six powders in the series were off-white in appearance and flowed freely, with the control powder slightly paler in colour in comparison to the CT containing powders. Spray-dried yields of the six dual loaded formulations investigated varied considerably (range: 60–90%, $n = 3$), with no evidence of a connection between formulation composition and spray-dried yield. However, spray-drying thermal efficiency increased with increasing CT chain length; this indicated higher heat absorption from higher molecular weight CT formulations (Fig. 1). The increase in thermal efficiency associated increased heat absorption potentially caused by the larger mass of higher molecular weight CT droplets/microspheres. The finite time of droplet residence in the drying chamber means heat–mass transfer must take place quickly, a larger mass absorbing more heat, reducing outlet temperatures.

HPLC analysis of drug loading revealed that all formulations, with the exception of the LMW/MMW formulation (84.1% of total dose), had a TS content within 5% of the expected total, with all BDP loads within 5% of the expect total (4%, w/w).

SEM images of the CT-modified spray-dried powders (Fig. 2B–F) indicated that the powders comprised regular spherical particles with a primary diameter of approximately 0.5–8 μm, and highlighted powder particle population poly-dispersity ($\sigma > 2$). The CT particulates appeared to have an undulating surface but most striking was the appearance of whisker-like material which littered the surface of particles. Increased particle corrugation has also been shown to decrease aggregation by reducing the surface area available to surface active forces (Chew et al., 2005a). The formation of the whiskers appeared to be independent of the CT molecular weight employed and they did not appear in the image of the control (Fig. 2A): this indicated that CT played a role in the production of the spears.

Intra-sample SEM variations in surface texture were observed. For example, the LMW/MMW formulation (Fig. 2C) showed a variety of textures with spiked particles next to smooth particles.

A yellowing of CT powders was noticed upon storage, yellowing was not seen in the control specimens. A similar phenomenon has been observed in samples of spray-dried morphine hydrochloride, where the researchers attributed the change to the thermal conditions of spray drying (Russo et al., 2006). Spray drying has been known to produce brilliant white powders (Seville et al., 2002, 2007a,b; Sham et al., 2004). A suggested aetiology of the observed discolouration of the spray-dried particulates in this study is the hydrolysis of lactose, which is white in colour, to glucose, which is yellow/brown in colour. The hydrolysis fuelled either by residual acetic acid left in the blend after the formation of the original chitosan gel, or by residual water content. The Maillard reducing sugar reaction between amino acids (LEU) and disaccharides (lactose) can be ruled out due to the standard storage conditions, short time frame and no evidence of change in the control (Klinkesorn et al., 2006). The change in colour of the CT powders did not appear to change flow property, and it was possible that the change in colour observed in the powders, that contained CT, may have been related to the formation of the whisker-like threads seen in the SEM imagery.

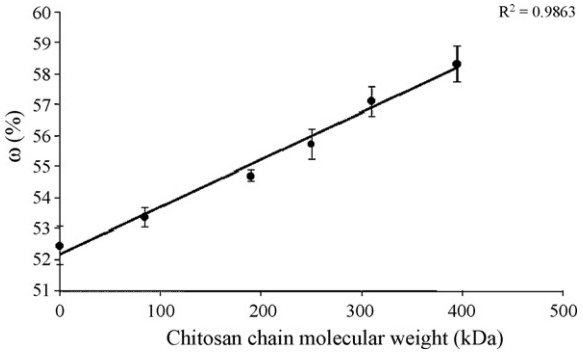

Fig. 1. Relationship of thermal efficiency (% ω) and median chitosan (CT) chain length in 50% (w/w) CT spray-dried powders (values are means ± S.D., $n = 3$).

DSC showed all six spray-dried formulations to be amorphous with no crystalline endothermic fusion peaks. Low water contents (range 0.5–5.5%, w/w) in the spray-dried powders were revealed, largely similar to the control (0.6%, w/w); the water content appeared to be independent of CT molecular weight (Table 2).

Dry dispersion Fraunhoffer size (volume mean diameter: volume mean diameter (VMD), range 3.4–9.1 μm) revealed a trend of increased LD size with increased CT molecular weight. Only two of the formulations were regarded as non-respirable based on VMD laser diffraction sizes (MMW/HMW and HMW; Table 2) (Bosquillon et al., 2004a). The dry dispersion Fraunhoffer sizes at 1 bar were likely to have included aggregates: so the increased particle size is likely to be associated with increased aggregate size; the relatively low vacuum pressure used for consistency with previous research in the field (Learoyd et al., 2008a,b). Comparison of the primary particle sizes exhibited in SEM images (Fig. 2) suggested that during particle sizing the CT-modified powders did not behave as individual particles, but rather as aggregates.

Spray-dried powder tapped densities remained consistent throughout the CT series (0.13–0.15 g cm^{-3}) – the control gave a slightly higher value (0.19 g cm^{-3}) – these values were in line with previous investigations (Rabbani and Seville, 2005). The tapped density of a formulation is associated with good aerosolisation; as more porous particles hold better aerodynamic property over solid particles of the same dimensions (Bosquillon et al., 2004b; Rabbani and Seville, 2005; Garcia-Arieta et al., 2001). Tapped density (p) gives an insight into the porosity of particles, the lack of a trend in tapped density across the CT series pointed to a greater degree of intra-particulate porosity (ϕ) in higher molecular weight CT spray-dried particles (Learoyd, 2007), which would be expected to have had greater crystalline density (p_c) pre-spray drying, as related to Eq. (4). Crystalline density was not tested as the crude CT was composed of semi-amorphous structures.

$$\phi = \frac{100(\rho_c - \rho)}{\rho_c} \quad (4)$$

The theoretical primary aerodynamic diameter (d_{ae}) of each formulation was calculated in triplicate and revealed primary particle diameters of 1.29–3.61 μm (LMW to HMW, respectively). Smaller theoretical particle sizes, in contrast to original laser diffraction sizes, indicated that the spray-dried particles behaved as aggregates rather than as individual microspheres during dry powder laser diffraction particle sizing (Table 2) (Bosquillon et al., 2004a).

It is also possible that the addition of CT gel to a formulation and the consequential increase in viscosity, in comparison to the control, could result in a larger droplet at the spray drying atomiser-fluid interface, which would spray-dry to give a larger particle—as previously hypothesised (Seville et al., 2007a; Rabbani and Seville, 2005; Bittner and Kissel, 1999).

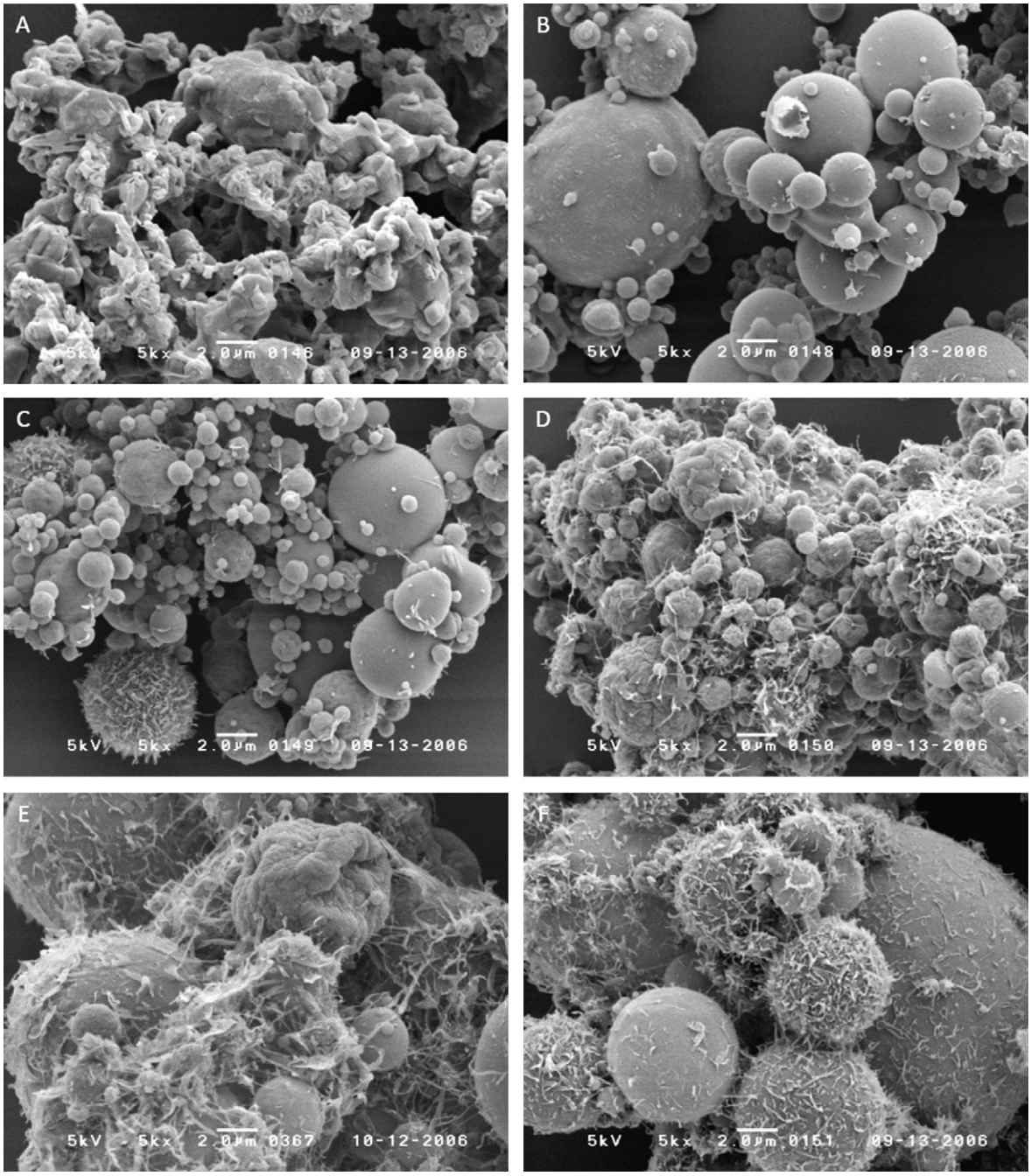

Fig. 2. Scanning microscopy images at 5 kV and 5000× magnification, formulations: (A) Control; (B) LMW CT; (C) LMW/MMW CT; (D) MMW CT; (E) MMW/HMW CT; (F) HMW CT.

Table 2
Physical characterisation of control and 50% (w/w) CT spray-dried powders (values are means ± S.D., $n = 3$).

Formulation	Water content (%)	Particle size (μm)	Tapped density (g cm^{-3})	Carr's index		d_{ae} (μm)
				(%)	Flowability	
Control	0.58 ± 0.15	3.42 ± 0.13	0.19 ± 0.02	23.03	Poor, fluid	1.47 ± 0.10
LMW	3.87 ± 0.12	3.36 ± 0.36	0.15 ± 0.01	37.96	Very poor	1.29 ± 0.14
LMW/MMW	5.50 ± 0.12	3.51 ± 0.08	0.14 ± 0.00	30.59	Poor, cohesive	1.30 ± 0.04
MMff	3.84 ± 0.06	4.63 ± 0.39	0.13 ± 0.00	31.92	Poor, cohesive	1.64 ± 0.14
MMWHMW	5.17 ± 0.23	8.20 ± 1.51	0.15 ± 0.01	22.71	Poor, fluid	3.12 ± 0.54
HMW	0.53 ± 0.16	9.08 ± 1.57	0.15 ± 0.00	30.44	Poor, cohesive	3.61 ± 0.52

LMW, 50% (w/w) low molecular weight CT; LMW/MMW, 25% (w/w) low and 25% (w/w) medium molecular weight CT; MMW, 50% (w/w) medium molecular weight CT; MMW/HMW, 25% (w/w) medium and 25% (w/w) high molecular weight CT; HMW, 50% (w/w) high molecular weight CT.

Table 3
Aerosolisation characterisation of control and CT spray-dried powders (values are means ± S.D., $n = 3$).

Formulation	ED (% TD)	FPF ($F < 5$ μm) (% TD)	MMAD (μm)
Control	98.56 ± 0.75	82.47 ± 2.64	2.04 ± 0.13
LMW	97.83 ± 0.92	75.83 ± 2.59	1.86 ± 0.22
LMW/MMW	98.40 ± 2.17	59.94 ± 2.11	2.75 ± 0.12
MMW	95.10 ± 4.07	56.86 ± 3.81	3.32 ± 0.28
MMW/HMW	98.04 ± 1.94	53.33 ± 2.73	4.05 ± 1.09
HMW	98.61 ± 0.11	51.53 ± 3.03	3.35 ± 0.18

ED: emitted dose; FPF: fine particle fraction; MMAD: mass median aerodynamic diameter.

Carr's index values represented the powder flow types as poor fluid to very poor (range 22.7–38.0%, Table 2); no correlation was observed between particle size, or visual flow properties, and representative Carr's index values.

3.2. In vitro powder aerosolisation

All powders showed high dispersibility, with at least 90% of the capsule contents emitted during aerosolisation testing (Table 3). Statistical analysis revealed no difference in the ED values ($P > 0.05$). Low deposition in the inhaler and throat regions of the MSLI was recorded, and indicated the highly dispersible nature of these powders. Individual stage deposition of both TS and BDP were not statistically different on any level of the MSLI for the dual loaded TS–BDP formulations, and showed homogeneity in the drug loading of individual microspheres and in the spray-dried formulations (Fig. 3; students paired t-test: non-significant, $P > 0.05$). The spray-dried formulations showed minimal deposition in the Spinhaler® device and the throat region of the MSLI. The control gave the lowest deposition of both TS and BDP on Stage 1 of the MSLI. The greatest deposition on Stages 3 and 4 of the MSLI was registered by the control, with the LMW formulation recording the greatest drug deposition on the terminal filter stage (Fig. 3).

A direct relationship between CT molecular weight and fine particle fraction was observed, with a reduction in the FPF (% total dose) with increased CT molecular weight (Table 3). The addition of CT to the TS–BDP formulation gave a significant decrease in FPF (control: LMW CT; $P < 0.05$), with the lowest FPF given by the HMW CT formulation (51.5%). Out of the formulations, LMW CT gave a highly significant increase in FPF compared to the other powders that contained CT ($P < 0.001$).

Overall there was a trend of improved aerosolisation with decreased CT molecular weight. The reduction in FPF with increased CT molecular weight may have been associated with increased CT molecular chain length, which increased hydrogen bonding/van der Waal sites for inter-particulate attraction. The aggregates that resulted were not dispersed by the force of inspiration into the MSLI (4 bar, via Spinhaler® DPI, 60 L min^{-1}). Furthermore, the decrease in FPF across the series with increased CT molecular weight could have been due to the decreased influence of LEU surface modification.

The average MMAD derived from TS and BDP MMAD mean values in the TS–BDP CT powders (range: 1.86–4.05 μm, Table 3) also showed a defined positive correlation with the theoretical and laser diffraction particle sizes: with a similar trend of increased particulate size with increased CT molecular weight. The increase in size attributed to the increased amount of chitin units (which make up CT) per unit area of liquid feed. As the tapped density of the spray-dried powders remained pretty much constant throughout the molecular weights, it can be deduced that the degree of porosity would increase throughout the series with increased CT molecular weight; this gave increased volume to individual microspheres and the formulation as a whole.

As particle size was related to CT chain length it also corresponded with increased thermal efficiency (ω). The relationship based on larger droplets, which produce larger primary particles and require higher energy to dry and hence reduce outlet temperatures and improve unit ω. An increase in dry dispersion LD size at 1 bar, due to aggregation, was also seen (Table 2).

Primary particle diameters also exhibited an increase in size: this thought to have been due to previously reported viscosity effects (Rabbani and Seville, 2005). MMAD size is influenced by both d_{ae} and aggregation, and was an intermediate of LD and d_{ae} results (Bosquillon et al., 2004b). Previous researchers have studied the influence and production of surface texture in the spray drying of surface textured particles (Chew et al., 2005b; Alexander and King, 1985); despite the absence of any pattern of coarse surface texture in the SEM images, surface corrugation may have impacted on the MMAD size increase over the series.

The relative good performance of the CT powders in the MSLI was principally attributed to high LEU concentrations. LEU has previously been shown to enhance a spray-dried formulation's aerosolisation properties—the solid state glidant activity of LEU previously used as a rational for any improvement in aerosolisation performance (Rabbani and Seville, 2005; Chew et al., 2005a; Li et al., 2005a,b; Najafabadi et al., 2004). Here the action of LEU in reducing surface active force is attributed to LEU particle fracture which produced rougher morphologies and reduced inter-particulate contact points for aggregation (Chew and Chan, 2001). The use of CT as a potential aerosolisation enhancer has also been explored with marginal success (Li and Birchall, 2006).

Another reason for decreased FPF across the series could have been the appearance of an increased number of strands on the SEM images with increased CT molecular weight: which could be

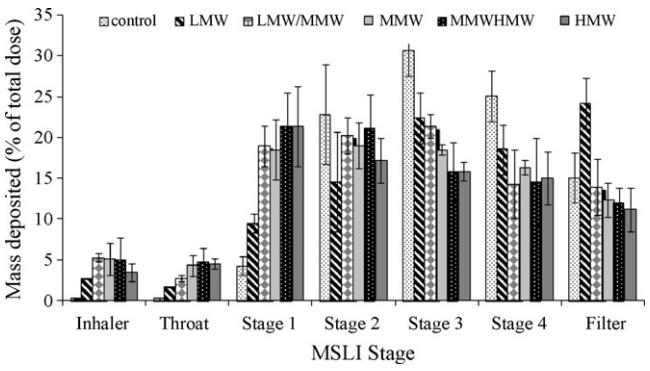

Fig. 3. Multi-Stage Liquid Impinger profile of the mean combined stage deposition of TS and BDP (values are means ± S.D., $n = 3$).

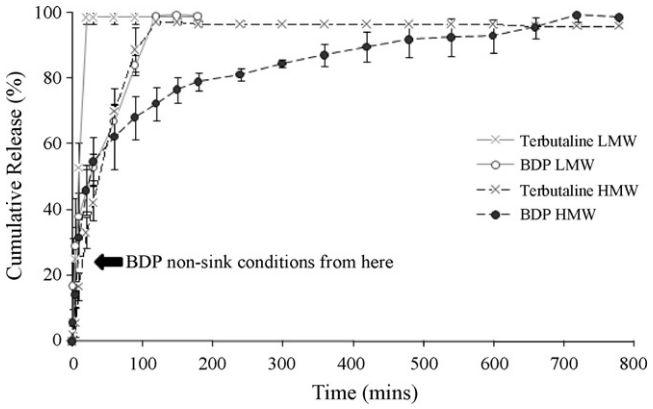

Fig. 4. Cumulative release of TS and BDP from LWM CT formulations and HWM CT formulations in rotating wire mesh baskets into 37 °C PBS (values are means ± S.D., $n = 3$).

Table 4
Dissolution kinetics of control and CT-modified spray-dried powders.

Formulation	t_{max} (mins)	Zero order		First order		Higuch matrix	
		t (min)	r^2	t (min)	r^2	t (min)	r^2
Control TS	5	0–5	0.97	0–5	0.98	0–5	0.99
Control BDP	30	0–30	0.84	0–30	0.86	0–30	0.96
LMW TS	30	0–30	0.95	0–30	0.75	0–60	0.85
LMW BDP	150	0–150	0.88	0–150	0.89	0–150	0.98
LMW/MMW TS	60	0–30	0.99	0–30	0.87	0–60	0.90
LMW/MMW BDP	180	0–180	0.82	0–180	0.96	0–180	0.96
MMW TS	60	0–30	0.99	0–30	0.90	0–60	0.97
MMW BDP	300	0–30	0.95	0–240	0.95	0–300	0.92
MMW/HMW TS	120	0–120	0.86	0–120	0.97	0–120	0.97
MMW/HMW BDP	360	0–120	0.75	0–240	0.95	0–360	0.94
HMW TS	120	0–120	0.95	0–120	0.94	0–120	0.98
HMW BDP	20	0–20	0.97	0–660	0.94	0–720	0.84

TS: terbutaline sulphate; BDP: beclometasone dipropionate.

a by-product of spray drying with CT, the strands may potentially increase aggregation and lower FPF (Fig. 2B–F).

3.3. *In vitro* dissolution

The CT powders produced a viscous gel upon contact with the phosphate buffer solution (PBS) and exhibited delayed release characteristics; increased CT molecular weight associated with a more sustained release profile. For example, the LMW powder released 100% TS after 30 min and 100% BDP after 150 min; whereas 2 h dissolution time was necessary for the HMW CT powder to release its entire TS drug load and 12 h to release the total BDP drug load (Fig. 4). The MMW CT powder displayed an intermediate release profile, with 100% drug release of TS after 60 min, 300 min for BDP.

The reason for the difference in the dissolution times of TS and BDP is the relative solubility of the two drugs in the dissolution media. TS is freely soluble in PBS so the diffusion down a concentration gradient to a more hydrophilic environment is rapid, as seen in the control; the physical obstruction of CT and any intermolecular attraction (hydrogen bonding, van der Waals forces) delayed the release of TS in the CT formulations (Chen et al., 2006a,b). BDP is only partially soluble in PBS; so the lipophilic corticosteroid slowly travelled down the concentration gradient. The CT gel therefore may have to swell to a greater extent to release BDP compared to the degree of swell necessary to deliver the TS fraction. Pre-hydration of the spray-dried samples presented a surface area of 7.4–7.9 mm^3 in the 2 cm diameter wire mesh baskets, visible expansion of the dry sample was observed up on contact with the dissolution media.

The application of the modified USP 2 dissolution apparatus to lung dissolution is tenuous: the formulation surface area post inhalation would be much greater as the preparation would not present as an accumulated mass of powder with a surface area of 7.4–7.9 mm^3. It would be expected that dissolution rates would also be affected by the reduced volume of dissolution media in the lung: the lung fluid lining is 6 mm in depth and differs greatly in its composition when compared to PBS (Hickey and Thompson, 1992). The dissolution method used in this study has previously been used in the research area (Learoyd et al., 2008a,c); however, no specific drug dissolution method to the lung existed at the point of study.

The fact that 100% BDP release was observed reveals that the hydrated CT gel did not provide a sufficiently hydrophobic environment for permanent BDP detainment. Following the 25% release of BDP from the matrix non-sink conditions were observed (Fig. 4), beyond which the BDP and TS profiles separated. TS affinity for the mobile phase and BDP saturation of the mobile phase may have caused the differences between TS and BDP after non-sink conditions for BDP dissolution were established. However, the prolonged release of BDP with increased CT chain length shows the ability of CT to modify BDP release irrespective of solution conditions.

In this study the release characteristics were modified through the use of low, medium and high molecular weight CT; the hypothesis was that the longer polymer chains in HMW CT would result in a more prolonged drug release profile compared to the lower molecular weight CT formulations, which comprise of shorter chains. Theories behind the differences in dissolution times for each molecular weight were based on CT chain length: the higher the molecular weight – the longer the individual fibres and the greater the hydrogen bonding, van der Waal forces and physical interlocking – the greater the drug entrapment and longer the period of dissolution (Asada et al., 2004; Chen et al., 2006a).

Comparison of the rates of release using zero and first order and the Higuchi homogeneous matrix rate equations revealed a dual release pattern (Table 4). An initial zero order burst phase was seen by all CT formulations, visualised as high correlation coefficients (r^2) related to the zero order kinetic from t_0 to early sample time points; the zero order kinetic possibly due to the presence of TS and BDP on the surface of primary powder particles. A secondary sustained release profile followed with the release TS and BDP drug molecules that resided within CT matrices: represented as high correlation between the Higuchi matrix kinetic and zero to high time points (Philip and Pathak, 2006). The presentation of Higuchi kinetics for both TS and BDP in the second phase of release from the CT indicates that the release of BDP during this stage is not simply due to the non-sink conditions after 25% cumulative release.

4. Conclusions

These investigations demonstrate that it was possible to generate highly respirable powders that exhibit sustained drug release profiles of both hydrophilic and lipophilic agents by spray drying. It was found that decreased spray-drying thermal efficiency, achieved through the use of a surfactant, correlated with the improved aerosolisation properties of a powder. *In vivo*, these powders would be predicted to deposit predominately in the central to peripheral region of the lung after inhalation, with minimal oropharyngeal deposition and reduced incidence of oropharyngeal side-effects. Once deposited in the lung, these powders would be anticipated to deliver the sustained release of both active agents, and offer the opportunity to reduce dose frequency and the number of formulations involved in a regimen.

Acknowledgements

This work has been financially supported through the provision of a Ph.D. studentship (T.P.L.) by the EPSRC and Pfizer Global Research and Development (CASE/CAN/04/06). The authors also

express their thanks to Dr. Gary Nichols, Pfizer, Sandwich for preparation of the SEM images.

References

Alexander, K., King, C.J., 1985. Factors governing surface-morphology of spray-dried amorphous substances. Dry. Technol. 3, 321–348.

Asada, M., Takahashi, H., Okamoto, H., Tanino, H., Danjo, K., 2004. Theophylline particle design using chitosan by the spray drying. Int. J. Pharm. 270, 167–174.

Balanag, V.M., Yunus, F., Yang, P.C., Jorup, C., 2006. Efficacy and safety of budesonide/formoterol compared with salbutamol in the treatment of acute asthma. Pulm. Pharmacol. Ther. 19, 139–147.

Barnes, N., 2006. Most difficult asthma originates primarily in adult life. Paediatr. Res. Rev. 7, 141–144.

Bittner, B., Kissel, T., 1999. Ultrasonic atomization for spray drying: a versatile technique for the preparation of protein loaded biodegradable microspheres. J. Microencapsul. 16, 325–341.

Black, S.D., Mould, D.R., 1991. Amino acid scale: hydrophobicity of physiological L-alpha amino acids. Anal. Biochem. 193, 72–82.

Bosquillon, C., Prat, W., Vanbever, R., 2004a. Pulmonary delivery of growth hormone using dry powders and visualization of its local fate in rats. J. Control. Release 96, 233–244.

Bosquillon, C., Rouxhet, P.G., Ahimou, F., Simon, D., Culot, C., Preat, V., Vanbever, R., 2004b. Aerosolization properties, surface composition and physical state of spray-dried protein powders. J. Control. Release 99, 357–367.

British National Formulary, 2007. BNF, 54th ed. RPS, London, p. 144.

Cazzola, M., Matera, M.G., 2008. Novel long-acting bronchodilators for COPD and asthma. Br. J. Pharmacol. 155, 291–299.

Chen, R., Okamoto, H., Danjo, K., 2006a. Particle design using a 4-fluid-nozzle spray-drying technique for sustained release of acetaminophen. Chem. Pharm. Bull. 54, 948–953.

Chen, R.C., Takahashi, H., Okamoto, H., Danjo, K., 2006b. Particle design of three-component system for sustained release using a 4-fluid nozzle spray-drying technique. Chem. Pharm. Bull. 54, 1486–1490.

Chew, N.Y.K., Chan, H.K., 2001. Use of solid corrugated particles to enhance powder aerosol performance. Pharm. Res. 18, 1570–1577.

Chew, N.Y.K., Tang, P., Chan, H.K., Raper, J.A., 2005a. How much particle surface corrugation is sufficient to improve aerosol performance of powders? Pharm. Res. 22, 148–152.

Chew, N.Y.K., Shekunov, B.Y., Tong, H.H.Y., Chow, A.H.L., Savage, C., Wu, J., Chan, H.K., 2005b. Effect of amino acids on the dispersion of disodium cromoglycate powders. J. Pharm. Sci. 94, 2289–2300.

Corrigan, D.O., Healy, A.M., Corrigan, O.I., 2006. Preparation and release of salbutamol from chitosan and chitosan co-spray dried compacts and multiparticulates. Eur. J. Pharm. Biopharm. 62, 295–305.

Dang, J.M., Leong, K.W., 2006. Natural polymers for gene delivery and tissue engineering. Adv. Drug Del. Rev. 58, 487–499.

Garcia-Arieta, A., Torrado-Santiago, S., Goya, L., Torrado, J.J., 2001. Spray-dried powders as nasal absorption enhancers of cyanocobalamin. Biol. Pharm. Bull. 24, 1411–1416.

Grenha, A., Seijo, B., Remunan-Lopez, C., 2005. Microencapsulated chitosan nanoparticles for lung protein delivery. Eur. J. Pharm. Sci. 25, 427–437.

Hardy, J.G., Chadwick, T.S., 2000. Sustained release drug delivery to the lungs—an option for the future. Clin. Pharmacokinet. 39, 1–4.

Harikarnpakdee, S., Lipipun, V., Sutanthavibul, N., Ritthidej, G.C., 2006. Spray-dried mucoadhesive microspheres: preparation and transport through nasal cell monolayer. Aaps Pharmscitech. 7.

Hickey, A.J., Thompson, D.C., 1992. Physiology of the Airways. Marcel Decker Inc., New York.

Holgate, S.T., Polosa, R., 2006. The mechanisms, diagnosis, and management of severe asthma in adults. Lancet 368, 780–793.

Holgate, S.T., Polosa, R., 2008. Treatment strategies for allergy and asthma. Nat. Rev. Immunol. 8, 218–230.

Huang, Y.C., Yeh, M.K., Cheng, S.N., Chiang, C.H., 2003. The characteristics of betamethasone-loaded chitosan microparticles by spray-drying method. J. Microencapsul. 20, 459–472.

Illum, L., 1998. Chitosan and its use as a pharmaceutical excipient. Pharm. Res. 15, 1326–1331.

Kim, J.C., Lee, H.Y., Kim, M.H., Lee, H.J., Kang, H.Y., Kim, S.M., 2006. Preparation and characterization of chitosan/gelatin microcapsules containing triclosan. Colloids Surfaces B: Biointerf. 52, 52–56.

Klinkesorn, U., Sophanodora, P., Chinachoti, P., Decker, E.A., McClements, D.J., 2006. Characterization of spray-dried tuna oil emulsified in two-layered interfacial membranes prepared using electrostatic layer-by-layer deposition. Food Res. Int. 39, 449–457.

Learoyd, T.P., 2007. Controlled Release in Inhalation Pharmacy. Aston University, Birmingham, p. 285.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006a. Influence of chitosan molecular weight on drug release from respirable spray-dried powders. J. Pharm. Pharmacol. 58, A15–A16.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2006b. Sustained drug release from respirable powders prepared by spraydrying w/o/w double emulsions containing PLGA, chitosan and leucine. J. Pharm. Pharmacol. 58, A67–A68.

Learoyd, T.P., Burrow, J.L., French, E., Seville, P.C., 2007. Controlled release formulations for pulmonary drug delivery. J. Pharm. Pharmacol. 59, A70–A170.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2008a. Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. Eur. J. Pharm. Biopharm. 68, 224–234.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2008b. Modified release of beclometasone dipropionate from chitosan-based spray-dried respirable powders. Powder Technol. 187, 231–238.

Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2008c. Modified release of beclometasone dipropionate from chitosan-based spray-dried respirable powders. Powder Technol. 187, 7.

Li, H.Y., Birchall, J., 2006. Chitosan-modified dry powder formulations for pulmonary gene delivery. Pharm. Res. 23, 941–950.

Li, H.Y., Seville, P.C., Williamson, I.J., Birchall, J.C., 2005a. The use of absorption enhancers to enhance the dispersibility of spray-dried powders for putmonary gene therapy. J. Gene Med. 7, 1035–1043.

Li, H.Y., Seville, P.C., Williamson, I.J., Birchall, J.C., 2005b. The use of amino acids to enhance the aerosolisation of spray-dried powders for pulmonary gene therapy. J. Gene Med. 7, 343–353.

Machado, J.J.B., Coutinho, J.A., Macedo, E.A., 2000. Solid-liquid equilibrium of alpha-lactose in ethanol/water. Fluid Phase Equilibr. 173, 121–134.

Martinac, A., Filipovic-Grcic, J., Voinovich, D., Perissutti, B., Franceschinis, E., 2005. Development and bioadhesive properties of chitosan-ethylcellulose microspheres for nasal delivery. Int. J. Pharm. 291, 69–77.

Masters, K., 1991. Spray Drying Handbook. John Wiley and Sons, New York.

Najafabadi, A.R., Gilani, K., Barghi, M., Rafiee-Tehrani, M., 2004. The effect of vehicle on physical properties and aerosolisation behaviour of disodium cromoglycate microparticles spray dried alone or with L-leucine. Int. J. Pharm. 285, 97–108.

Panigrahi, L., Pattnaik, S., Ghosal, S.K., 2005. The effect of pH and organic ester penetration enhancers on skin permeation kinetics of terbutaline sulfate from pseudolatex-type transdermal delivery systems through mouse and human cadaver skins. Aaps Pharmscitech. 6.

Philip, A.K., Pathak, K., 2006. Osmotic flow through asymmetric membrane: a means for controlled delivery of drugs with varying solubility. Aaps Pharmscitech. 7.

Rabbani, N.R., Seville, P.C., 2005. The influence of formulation components on the aerosolisation properties of spray-dried powders. J. Control. Release 110, 130–140.

Reid, M.L., Brown, M.B., Moss, G.P., Jones, S.A., 2008. An investigation into solvent-membrane interactions when assessing drug release from organic vehicles using regenerated cellulose membranes. J. Pharm. Pharmacol. 60, 1139–1147.

Russo, P., Sacchetti, C., Pasquali, I., Bettini, R., Massimo, G., Colombo, P., Rossi, A., 2006. Primary microparticles and agglomerates of morphine for nasal insufflation. J. Pharm. Sci. 95, 2553–2561.

Seville, P.C., Kellaway, I.W., Birchall, J.C., 2002. Preparation of dry powder dispersions for non-viral gene detivery by freeze-drying and spray-drying. J. Gene Med. 4, 428–437.

Seville, P.C., Learoyd, T.P., Li, H.Y., Williamson, I.J., Birchall, J.C., 2007a. Amino acid-modified spray-dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Powder Technol. 178, 40–50.

Seville, P.C., Li, H.Y., Learoyd, T.P., 2007b. Spray-dried powders for pulmonary drug delivery. Crit. Rev. Ther. Drug Carrier Syst. 24, 307–360.

Sham, J.O.H., Zhang, Y., Finlay, W.H., Roa, W.H., Lobenberg, R., 2004. Formulation and characterization of spray-dried powders containing nanoparticles for aerosol delivery to the lung. Int. J. Pharm. 269, 457–467.

Shaw, L.R., Irwin, W.J., Grattan, T.J., Conway, B.R., 2002. The development of a modified dissolution method suitable for investigating powder mixtures. Drug Dev. Ind. Pharm. 28, 1147–1153.

Sigurdsson, H.H., Loftsson, T., Lehr, C.M., 2006. Assessment of mucoadhesion by a resonant mirror biosensor. Int. J. Pharm. 325, 75–81.

Taki, M., Zeng, X.M., Oliver, M., Marriott, C., Martin, G.P., 2006. A comparison of the in-vitro deposition profiles of drugs from a combination dry powder inhaler (DIPI) using the Next Generation Impactor (NGI). J. Pharm. Pharmacol. 58, A65–A165.

Theophilus, A., Moore, A., Prime, D., Rossomanno, S., Whitcher, B., Chrystyn, H., 2006. Co- deposition of salmeterol and fluticasone propionate by a combination inhaler. Int. J. Pharm. 313, 14–22.