Research paper

Preparation and release of salbutamol from chitosan and chitosan co-spray dried compacts and multiparticulates

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Abstract

Chitosan microparticulates were prepared by spray drying from aqueous media containing hydrochloric acid or acetic acid. The medium affected the morphology and degree of acetylation of chitosan, the presence of acetic acid resulting in increased acetylation of the polymer during processing. Co-spray drying salbutamol sulphate/chitosan systems with the crosslinking agent formaldehyde had no detectable effect on particle morphology. However, with increasing salbutamol loading particles became less spherical, taking on a collapsed appearance. Spray dried chitosan–salbutamol sulphate microparticulates were X-ray amorphous. Chitosan–salbutamol sulphate composites were compressed into discs to quantify drug release and showed delayed release of salbutamol sulphate. The general power law equation fitted the data better than the $t^{0.5}$, mono- or bi-exponential models and gave n indices greater than 0.5, i.e. in the range 0.53–0.71. Crosslinking did not dramatically alter the drug release behaviour. Both crosslinked and non-crosslinked composites swelled during release, the former to the greater extent. The release data for crosslinked composites gave slightly higher n values than the corresponding non-crosslinked composites, consistent with the increased swelling of these systems. Release studies were also conducted on the microparticulates. Because of the small particle size and large surface area present, the release of the highly soluble drug salt was extremely rapid ($>90\%$ release in 5 min). Twin impinger analysis indicated good in vitro deposition of the microparticulates and potential for pulmonary delivery.
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Keywords: Chitosan; Salbutamol; Spray drying; Drug release

1. Introduction

Deacetylation of chitin yields the novel biomaterial chitosan. Chitins, which have been deacetylated to a degree of between 40 and 98%, are regarded as chitosan [1].

Paul and Sharma [2], Dodane and Vilivalam [3], Bernkop-Schnürch [4], Illum [1] and Khor and Lim [5] have published review articles on the potential pharmaceutical uses of chitosan. Paul and Sharma describe medical and pharmaceutical applications of chitosan which include drug delivery, wound healing ointments and dressings, artificial skin, haemostatic agents, enzyme immobilization, dialysis membranes, contact lenses or eye bandages. Genta et al. [6] described that the entrapment of dexamethasone in the chitosan

polymeric network using the spray drying method led to an improvement in dissolution rate of the drug, which did not occur in the equivalent physical mix. They attributed this to a reduction in crystallinity of the drug entrapped in chitosan in the spray-dried microspheres.

In contrast to using chitosan to aid dissolution, chitosan has been used for the preparation of sustained release systems. Nigalaye et al. [7] produced theophylline sustained release tablets containing chitosan, carbomer-93P and citric acid. Chitosan, when used at concentrations of greater than 50% of the tablet weight, resulted in an insoluble non-erosion type matrix. The induction period for drug release was increased with an increase in the degree of deacetylation of chitosan and with an increase in the amount of chitosan in the formulation. Dangprasirt and Pongwai [8] demonstrated the controlled release of diclofenac sodium from freeze–dried solid dispersions with ethylcellulose and chitosan as single and combination carriers. Giunchedi et al. [9] described the preparation and characterisation of ampicillin loaded chitosan and methylpyrrolidinone chitosan microspheres by a spray drying technique. Spray drying ampicillin with both chitosan and methylpyrrolidinone chitosan

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resulted in amorphous ampicillin. Nevertheless release of ampicillin from chitosan ampicillin microspheres was slower than dissolution of pure ampicillin. The activity of the antibiotic was not reduced by the spray drying technique.

Ganza-Gonzalez et al. [10] demonstrated the controlled release of metoclopramide when spray dried with chitosan using formaldehyde as a crosslinking agent. He et al. [11] spray dried chitosan base and chitosan hydrochloride alone with the drugs cimetidine, famotidine and nizatadine with and without the crosslinking agents formaldehyde and glutaraldehyde. Non-crosslinked chitosans swelled and dissolved in contact with water and crosslinking chitosan was found to be necessary to maintain the integrity of the microspheres. Crosslinking affected the particle size; as the amount of crosslinking agent increased the particle size decreased. The model drugs were shown by DSC to be molecularly dispersed within the chitosan. Loading was found to decrease with increasing amount of the crosslinking agent. The effect of changing molecular weight of chitosan on the release rate was negligible.

Tozaki et al. [12] investigated colon-specific insulin delivery with chitosan capsules. A 100% entrapment efficiency was obtained and release showed kinetics approaching zero order. Release rate could be increased by decreasing the chitosan content in the preparative solution.

Jameela and Jayakrishnan [13] prepared microspheres containing mitoxantrone and crosslinked chitosan and found drug release was controlled by the crosslinking, with only approximately 25% of drug release occurring over 36 days when microspheres with a high degree of crosslinking were analysed. Williams et al. [14] investigated microspheres made of chitosan as potential carriers for delivery of proteins, peptides and plasmid DNA to the lung via pressurised metered dose inhalers (MDIs). They investigated both non-crosslinked chitosan and crosslinked chitosan. They found non-crosslinked chitosan and glutaraldehyde-crosslinked chitosan microspheres to be potential candidates for carrying biotherapeutic compounds to the lung via MDIs due to their compatibility with the non-CFC propellant P134a and their favourable physicochemical characteristics. Carrión-Recio et al. [15] investigated chitosan microspheres prepared by a double emulsion microencapsulation technique as potential pulmonary delivery systems. They incorporated the model proteins insulin and bovine serum albumin and found the chitosan microspheres to have suitable morphological and aerodynamic properties for lung delivery.

The degree of deacetylation of chitosan affects the overall charge density, an increasing presence of ammonium groups resulting in a decrease in the crosslinking density related to hydrogen bonding and hydrophobic interactions [16]. An increase in the degree of deacetylation also results in increased swelling due to an increase in the number of ionic sites and their counter-ions [16].

The objective of the current work was to characterise and investigate the properties of chitosan composites prepared by spray drying as potential drug delivery systems for the lung. The release of the model drug salbutamol sulphate from co-spray dried crosslinked and non-crosslinked chitosan composites was investigated.

Salbutamol sulphate is used as a β_2-agonist in the treatment of asthma. Salbutamol sulphate is available in inhalation formulations as well as in oral and injectable formulations. It is a crystalline material, which is freely soluble in water and slightly soluble in ethanol [17]. Spray drying of salbutamol sulphate from aqueous solution results in an amorphous material [18].

2. Materials and methods

2.1. Materials

Salbutamol sulphate was kindly provided by IVAX Pharmaceuticals, Ireland, PEG 4000 was purchased from Riedel de Haën (Germany) and chitosan was purchased from Fluka (low molecular weight grade). Physical mixes were prepared using sub 63 µm mesh sieved powders mixed in a Turbula Mixer™ for 5 min.

2.2. Spray drying methodology

Chitosan and chitosan/salbutamol sulphate systems were spray dried using a Büchi 191 spray drier. Systems (crosslinked and non-crosslinked) were spray dried as 0.2% (w/v) solutions from 1% (v/v) acetic acid solutions using an inlet temperatures of 140 °C, pump setting of 15%, air flow rate of 600 l/h, aspirator set at 100% and outlet temperatures of 82–100 °C. Chitosan was also spray dried from HCl, as a 0.12% (w/v) solution from 0.8% (w/v) HCl using an inlet temperature of 140 °C, outlet temperature of 90–92 °C, aspirator rate of 100%, pump rate of 15% and airflow rate of 600 l/h.

2.3. Assessment of physicochemical properties

X-ray powder diffraction measurements (XRD) were made on samples using a Siemens D500 Diffractometer as previously described in Ref. [19].

Differential scanning calorimetry (DSC) was performed using a Mettler Toledo DSC 821^e as previously described in Ref. [19].

All spray dried systems were analysed by DSC and XRD within 1 h of production.

Scanning electron microscopy (SEM) was performed using a Hitachi S-3500N variable pressure scanning electron microscope.

Energy dispersive X-ray analysis (EDXA) was carried out using Tungsten Gamma Tech Imix-PTS EDX analysis on the Hitachi S-3500N variable pressure SEM with a 10 mm^2 UTW detector. For qualitative EDXA, powder samples were utilised and an area mapped for the presence of the atom being analysed.

HPLC analysis of salbutamol sulphate was performed using a variation of the USP Pharmacopeia method [20] for HPLC analysis of salbutamol sulphate, with sodium-1-heptane-sulfonic acid used in the preparation of the mobile phase instead of sodium-1-hexane-sulfonic acid, as previously described in Ref. [21].

Gel permeation chromatography (GPC) was performed using a Plaquagel-OH mixed 8 μm 300×7.5 mm column (Polymer Laboratories Ltd, UK). The analysis method supplied by Polymer Laboratories (Technical Bulletin TB581) was followed, substituting potassium nitrate for sodium nitrate. The mobile phase consisted of potassium nitrate 0.5 M and sodium phosphate monosodium 0.01 M brought to pH 2 with concentrated hydrochloric acid. The mobile phase flow rate was 1 ml/min. Samples were prepared as 0.05–0.10% (w/v) solutions in concentrated HCl and brought to volume with mobile phase. Pullulan polysaccharides (Polymer Laboratories) were used as standards and prepared as 0.05–0.10% (w/v) solutions in mobile phase. Two hundred micro liter volumes of samples or standards were injected in triplicate. The detector used was a Waters 410 differential refractometer. Analysis was performed using Shimadzu Class VP software (version 6.10) with GPC for Class VP (version 1.02).

2.4. Assay for degree of deacetylation of chitosan

The degree of deacetylation of chitosan was determined by one of two methods:

[1]H Nuclear magnetic resonance spectroscopy (^{1}H NMR)

Samples for ^{1}H NMR were prepared using the method of Hirai et al. [22]. Twenty milligram samples were dissolved in 2 ml of 2% (w/w) deuterated acetic acid in deuterated water solution. The solutions were heated to 70 °C to aid dissolution and filtered through cotton wool. Samples were analysed using a Bruker 400 MHz NMR. The degree of deacetylation was determined by Eq. (1) [22] using the integral intensity, I_{CH3}, of the CH_3 residue and the sum of integral intensities $I_{H2–H6}$ of the H_2, H_3, H_4, H_5, H_6' and H_6 protons.

$$\text{Degree of deacetylation}(\%) = \left\{ 1 - \left(\frac{1}{3} I_{CH_3} \Big/ \frac{1}{6} I_{H_2–H_6} \right) \right\} \times 100 \tag{1}$$

Ninhydrin assay

A ninhydrin colorimetric test [23] was used alternatively for the determination of the degree of deacetylation. Samples were analysed in triplicate.

2.5. In vitro release studies

These studies were performed using the USP 24 paddle apparatus [20]. Powders of a particle size less than 63 μm were used for preparation of non-spray dried discs and discs of physical mixes. The chitosan raw material was first milled (in a Retsch centrifugal mill with a 0.08 mm sieve ring attachment) prior to sieving through the laboratory test sieve (Endecotts Ltd). Powders, which were to be used in physical mixes, were mixed in a Turbula® mixer as described above. Discs of chitosan/salbutamol sulphate were prepared by weighing 150 mg of powder and compressing in a 13 mm punch and die set using a Perkin Elmer hydraulic press operated at 7 t for 5 min. Discs of pure crystalline salbutamol sulphate averaged

126.5 mg in weight. Dissolutions were carried out in a Sotax AT7 dissolution bath at 37 °C with paddles operating at 50 rpm. The dissolution medium used was 500 ml of Sørensens modified isotonic phosphate buffer pH 7 [24]. Five milliliter samples were taken, filtered through a 0.2 μm filter and replaced with 5 ml of fresh medium at 37 °C. Samples were analysed by HPLC as previously described in Ref. [21].

Salbutamol sulphate release from chitosan/salbutamol sulphate co-spray dried multiparticulates was performed using a variation of the method of Heelan and Corrigan [25]. The method involved adding 50 mg of powder to a 100 ml conical followed by the addition of 20 ml of isotonic phosphate buffer pH 7 (already heated to 37 °C). The conical was stoppered and placed into a horizontal shaker water bath maintained at 37 °C and shaken at 100 cycles/min. Two milliliter samples were withdrawn at appropriate time intervals and filtered through a 0.2 μm filter. Two milliliter of fresh medium at 37 °C was washed through the filter holder back into the conicals having first removed the filters. The filters were added into the conicals after each sample point to avoid loss of particles from the medium. Samples were analysed by HPLC as previously described [21].

2.6. Modelling drug release

The salbutamol release data were fitted to equations using MicroMath® Scientist® for Windows™ software. The ability of an equation to describe the experimental data was assessed using both the coefficient of determination (CoDet) and model selection criterion (MSC).

The MSC is a representation of the 'information content' of a given set of parameter estimates by relating the coefficient of determination to the number of parameters, i.e. the number of degrees of freedom used in obtaining the fit. Eq. (2) is used to determine the MSC.

$$\text{MSC} = \ln \left[\frac{\sum_{i=1}^{n} w_i (Y_{\text{obs}_i} - \bar{Y}_{obs})^2}{\sum_{i=1}^{n} w_i (Y_{\text{obs}_i} - Y_{\text{calc}_i})^2} \right] - \frac{2p}{n} \tag{2}$$

Where n is the number of points, w_i is the weight applied to each point, $\bar{Y}_{\text{obs}}$ is the weighted mean of the observed data, Y_{obs_i} is the observed values of dependent variable, Y_{calc_i} is the calculated values of dependent variable and p is the number of parameters estimated. When comparing two models, which have different numbers of parameters, the model selection criterion places the burden on the model with the greater number of parameters to have a better coefficient of determination. When judging the fit of a particular model to experimentally derived data, the model with the highest MSC is the most appropriate.

2.7. In vitro aerosol characterisation

In vitro deposition of dry powders for inhalation was determined using a twin impinger (Glaxo Type single stage

impactor, Copley Instruments (Nottingham) Ltd). The instrument complied with specifications for apparatus A of the European Pharmacopoeia [17] and British Pharmacopoeia [26]. Sixty milligram samples of powder were weighed and loaded into size 3 hard gelatin capsules [18], which were individually installed in a Rotahaler device (Glaxo Wellcome, Inc., NC). The Rotahaler was attached to the impinger which contained 7 and 30 ml of collecting solvent in stages 1 and 2, respectively. In the analysis of salbutamol/chitosan systems the collecting solvent used was acetic acid 1% (v/v), while in all other cases water was used as the solvent. The capsules contents were released by twisting the Rotahaler and the system was vacuumed to produce air streams of 60 l/min for 5 s. The liquids in stages 1 and 2 were collected and diluted as appropriate and measured by UV spectrophotometry or HPLC. Each deposition experiment involved the aerosolisation of one capsule. Respirable fraction was calculated as the amount deposited in the lower stage as a percentage of the emitted dose (amount emitted into upper and lower stages excluding the amount remaining in the device). All systems were analysed in triplicate. Statistical analysis was carried out using Minitab™ statistical software (Version 13.1).

Analysis of the salbutamol sulphate collected from the twin impinger apparatus was performed by HPLC as previously described in Ref. [21].

In the case of salbutamol sulphate systems (i.e. not co-spray dried), UV measurements were made using a Hewlett Packard 8452A photodiode array UV/vis spectrophotometer using 10 mm quartz cuvettes (Spectrosil). Absorbances were measured at 276 nm for salbutamol sulphate analyses. Concentrations were determined by reference to a calibration curve prepared from dilutions of stock solutions of salbutamol sulphate.

3. Results and discussion

3.1. Spray drying of chitosan

Analysis by SEM showed that spray-drying chitosan reduced the particle size and changed the particle morphology. The particle morphology depended on the solvent from which the chitosan was spray dried. SEM micrographs are shown in Fig. 1. Spray drying from acetic acid solution resulted in uniform spherical particles, which had a roughened folded surface with diameters ranging from 1 to 4 μm, the particles being similar in morphology and particle size to those reported by He et al. [11].

XRD scans of chitosan starting material and processed chitosans are shown in Fig. 2. All systems showed diffuse halos with two regions of short-range order. Zong et al. [27] describe XRD scans of chitosan raw material and a chitosan cast film prepared from its solution in water/acetic acid. In contrast to the amorphous materials obtained in the current work, they describe two crystal forms of chitosan, form I showing strongest reflection at 10° 2θ and form II showing strongest reflection at 20° 2θ by XRD, both forms being orthorhombic. Production of the cast film resulted in a change in the relative
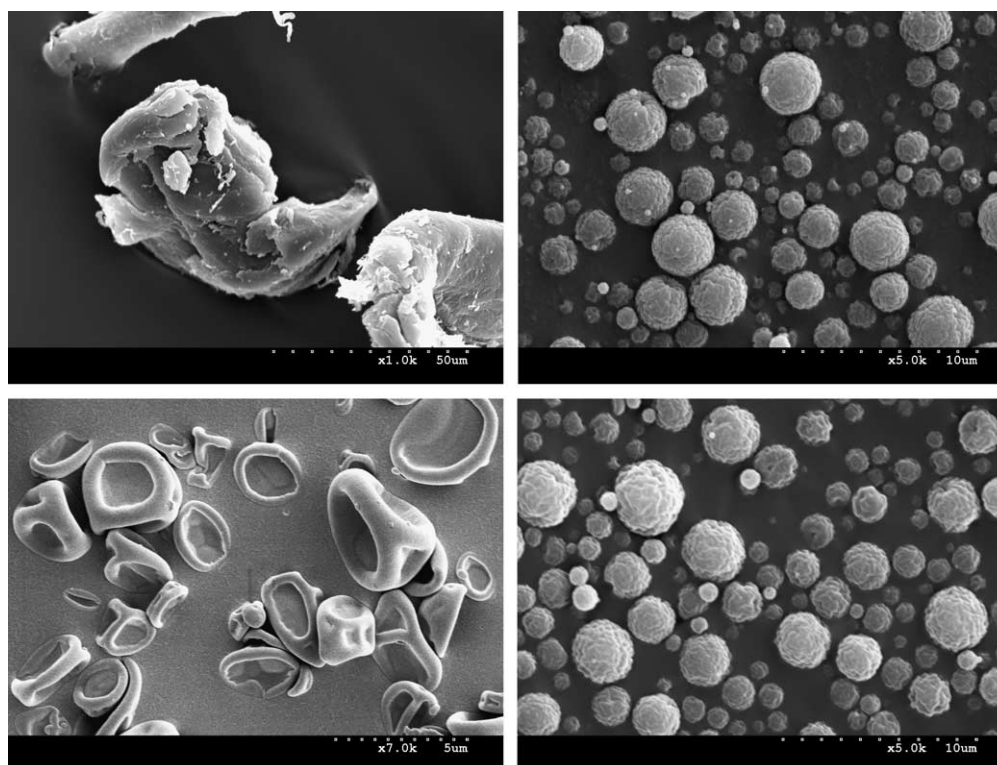

Fig. 1. SEM micrographs of milled chitosan (top left); chitosan spray dried from acetic acid (top right); chitosan spray dried from HCl (bottom left) and crosslinked chitosan spray dried from acetic acid (bottom right).
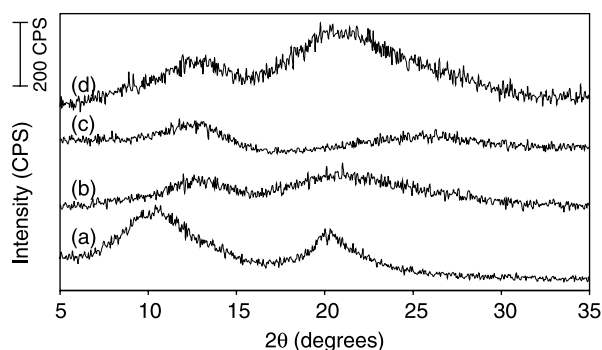
Fig. 2. XRD scans of (a) chitosan starting material; (b) chitosan spray dried from 1% (v/v) acetic acid; (c) chitosan spray dried from 0.8% (w/v) HCl and (d) crosslinked chitosan spray dried from 1% (v/v) acetic acid.

intensity of the peaks at 20° and 10° 2θ indicating a partial change from crystal form II to form I.

DSC scans of chitosan starting material and spray dried chitosan are shown in Fig. 3.

No sharp endotherms were visible. The unprocessed chitosan showed only one broad endotherm. All other chitosans showed two broad endotherms. Lee et al. [28] proposed that although chitosan has crystalline regions, the crystalline melting temperature is not found because of the rigid-rod polymer backbone having strong inter and/or intra molecular H-bonding. Kim et al. [29] describe this behaviour for chitin and hydroxypropyl chitin. They state that rigid-rod-type polymers show glass transitions followed by rapid thermal degradation and that in general DSC does not provide information of glass transition temperatures or melting temperatures of polysaccharides. Using dynamic mechanical analysis they found glass transitions of chitin at 236 °C.

Crosslinking chitosan did not change the particle shape or surface characteristics and resulted in particles of a similar size

range as the non-crosslinked chitosan spray dried from acetic acid. Spray drying from HCl resulted in concave collapsed particles with smooth surfaces. The longest dimension of these particles was approximately 5 μm.

Two different batches of chitosan raw material were initially examined. Chitosan is obtained from the deacetylation of chitin, which is obtained from natural sources, and therefore batch-to-batch variability can be high. Hence, both batches were characterised with respect to molecular weight and degree of deacetylation.

Similar number average (165,595 and 164,651) and weight average molecular weights (837,375 and 643,611) were obtained using GPC for chitosan raw materials and no significant change in molecular weight was observed on spray drying these batches from aqueous 1% (v/v) acetic acid. However, chitosan samples spray dried from HCl showed much lower number (26,298) and weight average molecular weights (78,574). A suitable solvent for the preparation of crosslinked chitosan solutions for GPC could not be found, crosslinked chitosans being insoluble in acetic acid, aceto-nitrile and tetrahydrofuran.

The degree of deacetylation affects the physicochemical properties of chitosan such as solubility and viscosity of chitosan solutions [1]. The effect of spray drying on the degree of deacetylation was investigated. The degree of deacetylation was initially determined by ninhydrin assay (which detects the –NH$_2$ group on the glycoside repeating unit of chitosan) and also by ^{1}H NMR. Ninhydrin assay gave consistently lower values for the degree of deacetylation than ^{1}H NMR (50% versus 82%). This difference between ninhydrin and ^{1}H NMR has been previously described in the literature with the higher results obtained using ^{1}H NMR being generally recognised as the more accurate [30]. Since ninhydrin analysis gave lower values for the degree of deacetylation of chitosan raw material than that described by
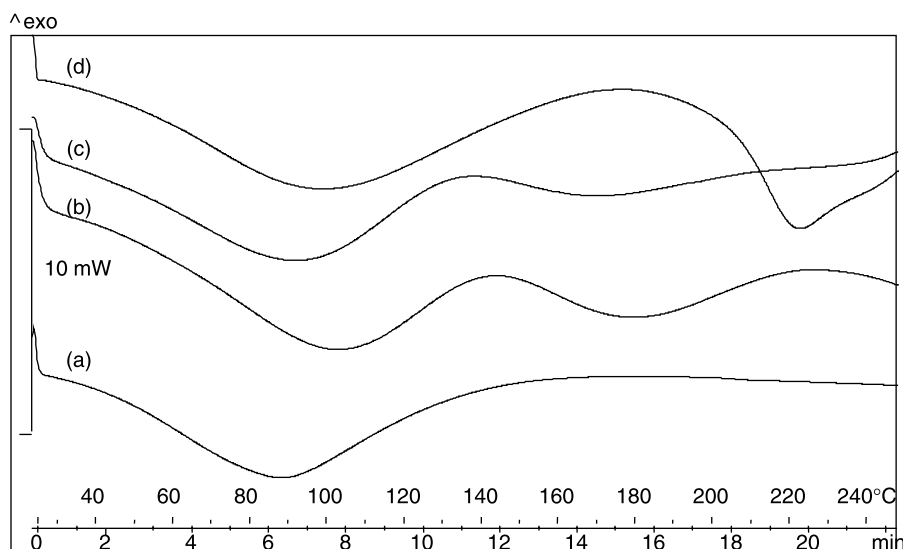

Fig. 3. DSC scans of (a) chitosan; (b) chitosan spray dried from 1% (v/v) acetic acid solution; (c) chitosan spray dried from 0.8% (w/v) hydrochloric acid solution and (d) crosslinked chitosan spray dried from 1% (v/v) acetic acid.

the manufacturer's specifications, ^{1}H NMR was adopted as the method of choice for determining degree of deacetylation in this work. The two different batches of chitosan purchased from Fluka, were stated to have a degree of deacetylation of 75–85%. When analysed by ^{1}H NMR they were both found to have a degree of deacetylation of 82%. Spray drying chitosan from acetic acid resulted in a reduction in the degree of deacetylation. Deacetylation values of 50 and 55% (for batches 1 and 2 of raw material) were obtained by ^{1}H NMR. Chitosan spray dried from 0.8% (w/v) HCl was shown to have a degree of deacetylation of 79% by ^{1}H NMR, no significant change from unprocessed chitosan, which gave average deacetylation values of 82%. These results indicate that spray drying chitosan from acetic acid results in a reduction in the degree of deacetylation unlike spray drying from 0.8% (w/v) HCl solution. It is likely that chitosan spray dried from acetic acid forms some chitosan acetate and therefore the amide groups are no longer available for detection. Crosslinking chitosan during spray drying resulted in a dramatic reduction in the degree of free amino groups measured as degree of deacetylation. A value of 1% deacetylation (in fact 1% free amino groups) was obtained for crosslinked chitosan by ^{1}H NMR analysis. Non-crosslinked chitosan is water soluble as the salt with various acids on the amino group of the D-glucosamine unit [31]. Crosslinking chitosan removes the functional group necessary for salt formation thus reducing chitosans ability to form water-soluble salts.

3.2. Salbutamol sulphate/chitosan systems (not crosslinked)

Spray dried salbutamol sulphate/chitosan systems containing 10, 20 and 40% salbutamol sulphate were shown to be amorphous by XRD with two regions of short range order. The DSC scans showed two broad endotherms similar to the DSC scan obtained for chitosan alone. The physical mix shows a salbutamol sulphate melting endotherm peaking at approximately 205 °C. No such melting endotherms are visible in this region for the spray-dried systems. He et al. [11] spray dried chitosan with cimetidine and famotidine with and without crosslinking agent and found no endothermic peaks indicative of melting of either drug. They concluded that the lack of endotherms for the drug indicated that the drugs were dispersed inside the matrix of chitosan as a solid solution. Since DSC of salbutamol sulphate/chitosan co-spray dried systems showed no sharp endotherms indicative of melting of crystalline material, and XRD also showed no peaks indicative of crystallinity, the salbutamol sulphate/chitosan co-spray dried systems exist as glass solutions.

SEM micrographs of salbutamol sulphate/chitosan spray dried composites are shown in Fig. 4. SEM micrographs of the 10 and 20% salbutamol sulphate systems showed particles with a similar morphology to chitosan spray dried alone, with diameters of approximately 1–4 µm. Particles were spherical with a roughened surface. The 20% salbutamol sulphate particles showed some signs of collapsing, with surface indentations apparent. SEM of the 40% salbutamol sulphate/
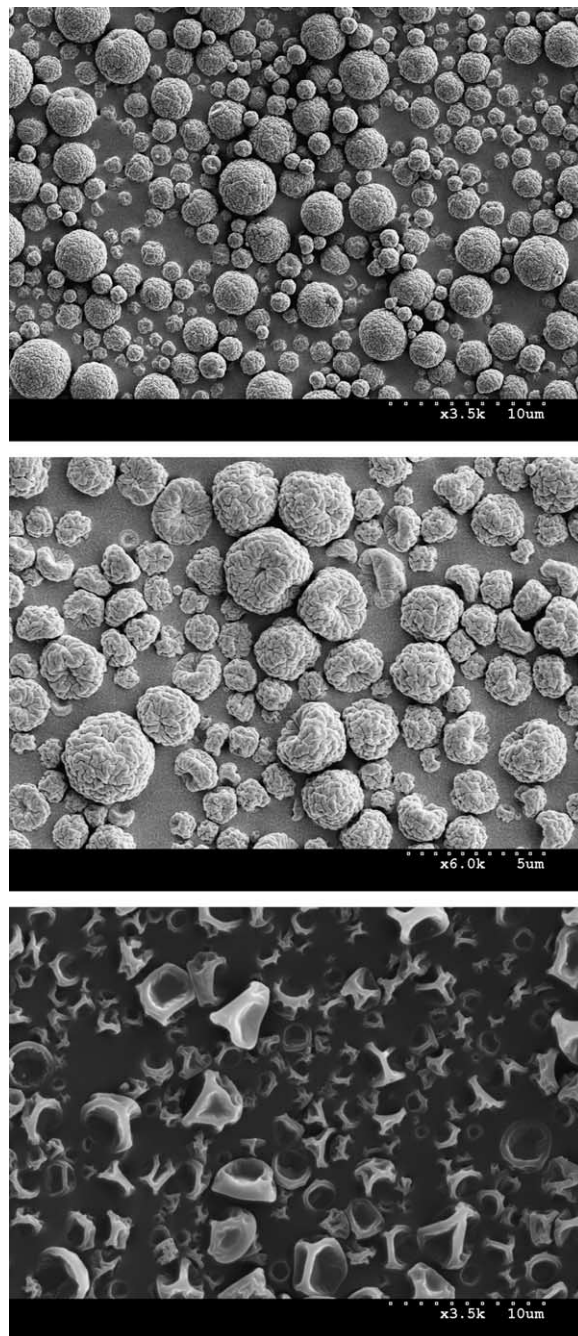

Fig. 4. SEM micrographs of spray dried 10% salbutamol sulphate/chitosan (top); 20% salbutamol sulphate/chitosan (middle) and 40% salbutamol sulphate/chitosan (bottom).

chitosan system showed particles of a different morphology. They were collapsed but smooth surfaced, the lengths of the largest particles being approximately 5 µm.

Due to the low solubility of chitosan the co-spray dried systems were spray dried at low feed concentration, i.e. feed concentrations of 0.2% (w/v). Feed concentration is a major factor in determining particle size of the resultant powders. The difference in size and size distribution for different feed concentrations was demonstrated previously by Corrigan et al. [21] by spray drying salbutamol sulphate/lactose 20% at two

different feed concentrations. When the SEM micrographs of the two systems were compared it was apparent that a change in feed concentration from 10 to 2.5% (w/v) could result in the production of smaller diameter particles with a narrower size distribution.

To determine whether the salbutamol present in the co-spray dried salbutamol sulphate/chitosan composites remained in the sulphate form, quantitative EDXA was performed on discs prepared from physical mixes and spray dried salbutamol sulphate/chitosan systems. Results showed no difference in sulphur content between spray dried and physical mix systems, consistent with no change in the salt form occurring on spray drying.

3.3. Crosslinked salbutamol sulphate/chitosan systems

The XRD scans of 10, 20 and 40% salbutamol sulphate/chitosan systems which were spray dried with the crosslinking agent, formaldehyde, indicated that these systems were amorphous. Each system showed two regions of short-range order. The DSC scans were similar to spray dried non-crosslinked chitosan/salbutamol sulphate systems.

SEM micrographs of spray dried salbutamol sulphate/crosslinked chitosan systems had similar morphologies to the corresponding spray dried non-crosslinked systems (Fig. 4). Particle size ranges were also similar to non-crosslinked salbutamol sulphate/chitosan spray dried systems.

3.4. In vitro release of salbutamol sulphate from salbutamol sulphate/chitosan systems

3.4.1. In vitro release from compressed discs

To determine the likely ability of chitosan to retard drug release, and the possible mechanisms involved in such drug release, compressed discs were prepared in order to control the surface area. Discs were prepared from 10, 20 and 40% salbutamol sulphate/chitosan co-spray dried systems produced with and without crosslinking agent. Discs were also prepared from physical mixes of 40% crystalline salbutamol sulphate and either 60% spray dried chitosan produced with or without crosslinking agent or milled chitosan. Morimoto et al. [32] used phosphate buffered saline pH 7 when investigating in vitro release of salmon calcitonin from microspheres for potential pulmonary delivery. Nielson et al. [33] described the alveolar subphase pH in the lungs of anesthetized rabbits as 6.92. Therefore, isotonic phosphate buffer pH 7 was chosen as the dissolution medium for the in vitro salbutamol sulphate/chitosan release experiments. Co-spray dried systems and physical mixes of salbutamol sulphate with spray dried chitosan gave considerably slower release than discs prepared from pure drug alone or discs prepared from physical mixes of salbutamol sulphate and milled chitosan. The results are shown in Fig. 5. Release was complete for most systems by 100 min with all systems showing complete release by 210 min. The amount of drug released was in proportion to the amount present in the mixes.

The experimental release profiles of salbutamol sulphate/chitosan systems were initially fitted to the square
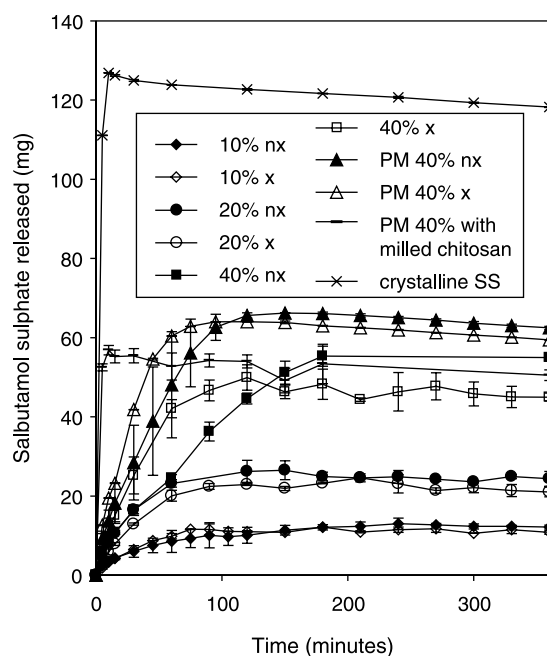
Fig. 5. Release of salbutamol sulphate from compressed discs ($n = 3$). 10% *nx*, spray dried not crosslinked 10% salbutamol sulphate/chitosan; 10% *x*, spray dried crosslinked 10% salbutamol sulphate/chitosan; 20% *nx*, spray dried not crosslinked 20% salbutamol sulphate/chitosan; 20% *x*, spray dried crosslinked 20% salbutamol sulphate/chitosan; 40% *nx*, spray dried not crosslinked 40% salbutamol sulphate/chitosan; 40% *x*, spray dried crosslinked 40% salbutamol sulphate/chitosan; PM 40% *nx*, physical mix of 40% crystalline salbutamol sulphate/spray dried not crosslinked chitosan; PM 40% *x*, physical mix of 40% crystalline salbutamol sulphate/spray dried crosslinked chitosan; PM 40% with milled chitosan, physical mix 40% crystalline salbutamol sulphate/milled chitosan; SS, salbutamol sulphate.

root of time relationship (Eq. (3)) [34] reflecting diffusion from a homogeneous matrix involving diffusion through the matrix into the surrounding medium.

$$W = kt^{0.5} \tag{3}$$

where W is the mass of drug released at time t per unit exposed area and k is a constant, $\sqrt{2DAC_s(\varepsilon/\tau)}$ when C_s is much less than A. D is the diffusivity of the drug in the homogeneous matrix media, A is the total amount of drug present in the matrix per unit volume and C_s is the solubility of the drug in the matrix substance.

Since the equation was derived from the flux from an infinite planar matrix, the equation will only remain valid for approximately 60% release from a cylinder. The salbutamol sulphate release data was fitted to the equation (Fig. 6). The k value, obtained from fitting the salbutamol sulphate release from salbutamol/chitosan spray dried systems to the Higuchi model, increased with salbutamol sulphate loading (A). For both the 10 and 40% systems the crosslinked systems tended to give higher k values. Higher values were also obtained from discs prepared from physical mixes of salbutamol sulphate with spray dried crosslinked chitosan than physical mixes with non-crosslinked chitosan. Table 1 shows the k values as well as the MSC and coefficient of determination values obtained for the fitting of the salbutamol sulphate release data. The data did
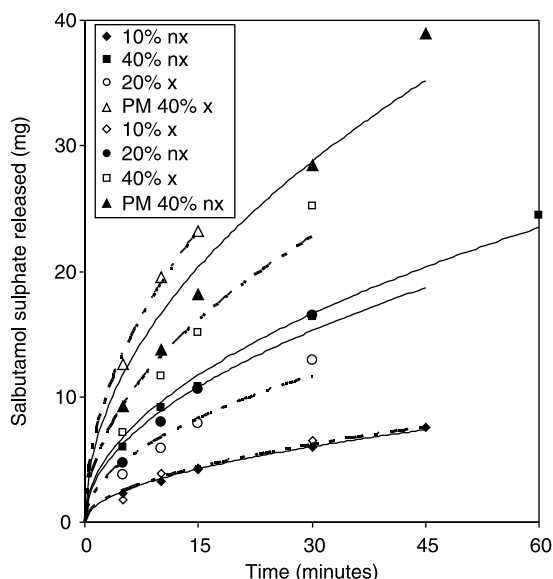
Fig. 6. Salbutamol sulphate release data from salbutamol sulphate/chitosan systems fitted to the Higuchi model. 10% *nx*, spray dried not crosslinked 10% salbutamol sulphate/chitosan; 10% *x*, spray dried crosslinked 10% salbutamol sulphate/chitosan; 20% *nx*, spray dried not crosslinked 20% salbutamol sulphate/chitosan; 20% *x*, spray dried crosslinked 20% salbutamol sulphate/chitosan; 40% *nx*, spray dried not crosslinked 40% salbutamol sulphate/chitosan; 40% *x*, spray dried crosslinked 40% salbutamol sulphate/chitosan; PM 40% *nx*, physical mix of 40% crystalline salbutamol sulphate/spray dried not crosslinked chitosan; PM 40% *x*, physical mix of 40% crystalline salbutamol sulphate/spray dried crosslinked chitosan. —, fitted non-crosslinked; ▬·▬·, fitted crosslinked.

not fit the equation particularly well with model selection criterion values (MSC) generally below three. The Higuchi model assumes no change in the dimensions of the matrix during drug release. During the chitosan/salbutamol sulphate release studies the discs were observed to swell by 1.2–1.6 times their original diameters.

Schwartz et al. [35] found the release of drug into aqueous media from wax matrices to give acceptably linear first order semi-log plots with slopes that were apparently independent of the concentration of drug embedded in the wax matrix. Therefore, the data was fitted to the first order equation

shown in Eq. (4).

$$W = W_\infty(1 - e^{-kt}) \tag{4}$$

where W is weight released at time t, W_∞ is the weight released at infinity and k is the release rate constant. From Fig. 5 it is apparent that complete release has occurred by 210 min therefore only data up to and including time point 210 min was used for fitting to the first order model. The parameter estimates obtained and related statistics are summarized in Table 2. The physical mixes were fitted by fixing the weight released at infinity to 60 mg, the total amount present. The rate constant k is reasonably similar for all systems, again the crosslinked 10, 40% and physical mix systems showed higher k values than the corresponding non-crosslinked systems (Table 2). For both the 10 and 20% systems, less than 100% drug was released by the time the profiles reached a plateau. Overall the fit was better, average MSC values being 3.5 compared to 2.9 for the Higuchi fit.

From Fig. 5 it appeared that there was a tendency for profiles to peak and decline, which could reflect degradation of the drug over time. Therefore, the data was fitted to Eq. (5), an equation describing the difference between two exponentials, one representing release of the drug and the other representing degradation of the drug.

$$W = W_m(e^{-K_D t} - e^{-K_R t}) \tag{5}$$

where W is the weight released at time t, W_m is the total weight released, k_D is the degradation rate constant and k_R is the release rate constant. The release rate constants, k_R were quite similar to the rate constant, k, in the first order fitted data (Table 2). There was no significant improvement over the first order fit when the average MSC values were compared.

An alternative approach to the modelling of polymeric systems is the 'general power law expression' described by Sinclair and Peppas [36] and Ritger and Peppas [37]. This model was developed to model transport in glassy polymers and therefore was considered applicable to our systems. The model is only considered valid for up to 60% release and the equation for weight released (W) may be written as follows:

$$W = W_m k t^n \tag{6}$$

Table 1
Model parameter estimates and related goodness of fit statistics (MSC is the model selection criterion and CoDet is the coefficient of determination) for salbutamol sulphate release data fitted to the general power law expression and the Higuchi model (abbreviations are as described in Fig. 7)

System	General power law expression $W = k^l t^n$				Higuchi model $W = kt^{0.5}$		
	k^l (mg/min^n)	n	MSC	CoDet	K (mg/min$^{0.5}$)	MSC	CoDet
10% *nx*	0.9854	0.5330	5.520	0.998	1.0994	4.918	0.995
10% *x*	0.8361	0.6048	2.286	0.963	1.1343	2.263	0.937
20% *nx*	1.6830	0.6725	5.659	0.999	2.7866	2.318	0.940
20% *x*	1.1720	0.7051	6.963	1.000	2.1358	2.084	0.925
40% *nx*	2.3850	0.5680	6.094	0.999	3.0313	3.935	0.987
40% *x*	2.2560	0.7080	6.384	0.999	4.1544	2.058	0.923
PM 40% *nx*	2.8834	0.6800	5.401	0.998	5.2480	2.452	0.942
PM 40% *x*	3.6890	0.7090	3.374	0.987	5.9900	3.520	0.985
Average			5.210	0.993		2.944	0.954

Table 2
MicroMath® Scientist® goodness of fit and parameter values for release data fitted to the first order and the biexponential model

System	1st order Model $W = W_\infty(1-e^{-kt})$				Biexponential Model $W = W_m(e^{-k_Dt} - e^{-k_Rt})$				
	k (min^{-1})	W_∞	MSC	CoDet	k_D (min^{-1})	k_R (min^{-1})	W_m	MSC	CoDet
10% nx	0.0239	11.54	2.894	0.959	$2.52e^{-19}$	0.0204	12.23	2.947	0.962
10% x	0.0321	11.55	3.435	0.976	0.00031	0.0296	12.23	3.433	0.977
20% nx	0.0363	25.69	4.508	0.993	0.00038	0.0335	27.37	4.655	0.994
20% x	0.0284	23.67	4.400	0.992	0.00059	0.0254	26.15	4.170	0.989
40% nx	0.0115	58.97	3.451	0.979	$8.7e^{-5}$	0.0114	60	3.484	0.979
40% x	0.0285	48.32	3.602	0.982	0.00053	0.0250	53.52	4.049	0.988
PM 40% nx	0.0277	60	2.768	0.990	$4.82e^{-19}$	0.0277	60	2.651	0.944
PM 40% x	0.0435	60	3.175	0.988	$4.82e^{-19}$	0.0435	60	3.057	0.962
Average	0.029		3.529	0.982	0.000271	0.0271		3.56	0.97

MSC is the Model Selection Criterion and CoDet is the Coefficient of Determination. (Abbreviations are as described in Fig. 7.)

where W_m is the total drug present, k is the diffusional constant and n is the diffusional exponent. The release data was fitted to Eq. (6) and is shown in Fig. 7. If drug release was matrix diffusion controlled (Fickian) then a value of 0.5 would be obtained for n in Eq. (6). Values of greater than 0.5 indicate anomalous diffusion [37]. This is generally indicative of a system that swells. The closer the value obtained is to 1, the closer the release is to zero order. Eq. (6) gave a better fit for the salbutamol sulphate release data than any of the other models previously described, the average MSC being 5.2. The values of n were greater than 0.5 and in all cases the value of n was higher for the crosslinked than non-crosslinked equivalent system. These higher values for n for the crosslinked discs are consistent with greater swelling during release (18–24%). The diffusional constant, k, tended to increase with salbutamol sulphate loading. Tables 1 and 2 show a summary of the statistics and parameter estimates for the release profiles fitted to the models discussed.

The reason for the increased swelling of the crosslinked chitosan over the non-crosslinked chitosans is unclear. Chitosans exhibit a high level of inter and intra-molecular hydrogen bonding. It is likely that crosslinking with formaldehyde disrupts some of the hydrogen bonding that is present in non-crosslinked material.

3.4.2. In vitro release from microparticles

Release experiments were carried out for the 10% crosslinked and non-crosslinked spray dried salbutamol sulphate/chitosan systems. The release was very rapid in both cases. Greater than 90% of salbutamol sulphate was released from the non-crosslinked salbutamol sulphate/chitosan microparticles in the first 5 min and 100% release occurred within 5 min for the crosslinked microparticles. In light of the previous disc studies this is not surprising. In the case of diffusion from the discs the maximum distance was 450 μm (discs were 0.9 mm in depth). In contrast the average diffusional distance in the particles was 0.8 μm (average radius of 100 particles). Therefore, the diffusional distance in the microparticle system was approximately 563 times shorter than in the disc systems. The half life of release from the particles was approximately 2.5 min for the crosslinked system and just

over 2.5 min for the non-crosslinked system. There was no significant difference between release from crosslinked versus non-crosslinked systems.

3.5. In vitro deposition of microparticles (twin impinger studies)

The results of the twin impinger studies indicate that the co-spray drying process leads to powders with inhalable fractions. The salbutamol sulphate/chitosan co-spray dried systems gave high respirable fractions, with no significant differences existing between crosslinked and non-crosslinked systems or between systems of different weight ratios of salbutamol
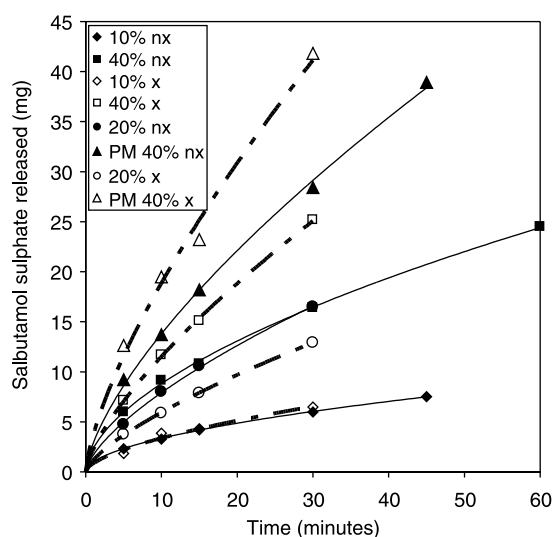

Fig. 7. Salbutamol sulphate release data from salbutamol sulphate/chitosan systems fitted to the general power law expression. 10% nx, spray dried not crosslinked 10% salbutamol sulphate/chitosan; 10% x, spray dried crosslinked 10% salbutamol sulphate/chitosan; 20% nx, spray dried not crosslinked 20% salbutamol sulphate/chitosan; 20% x, spray dried crosslinked 20% salbutamol sulphate/chitosan; 40% nx, spray dried not crosslinked 40% salbutamol sulphate/chitosan; 40% x, spray dried crosslinked 40% salbutamol sulphate/chitosan; PM 40% nx, physical mix of 40% crystalline salbutamol sulphate/spray dried not crosslinked chitosan; PM 40% x, physical mix of 40% crystalline salbutamol sulphate/spray dried crosslinked chitosan. —, fitted non-crosslinked; — · ·, fitted crosslinked.

Table 3

Mean respirable fractions (respirable percentage) and standard deviations of salbutamol sulphate from capsules of salbutamol sulphate micronised raw material, Ventolin Rotacaps® and capsules of co-spray dried salbutamol sulphate/chitosan composites, obtained using the twin impinger apparatus

System	Mean respirable % ($n=3$)	Standard deviation ($n=3$)
Micronised salbutamol sulphate	1.54	0.29
Ventolin Rotacaps®	39.97[a]	1.86[a]
Spray dried 10% salbutamol sulphate/chitosan	28.41	1.70
Spray dried 20% salbutamol sulphate/chitosan	32.01	1.40
Spray dried 40% salbutamol sulphate/chitosan	32.99	10.40
Spray dried 10% salbutamol sulphate/crosslinked chitosan	36.13	7.04
Spray dried 20% salbutamol sulphate/crosslinked chitosan	29.29	4.82
Spray dried 40% salbutamol sulphate/crosslinked chitosan	36.46	4.10

[a] ($n=2$).

sulphate to chitosan (Table 3). Respirable fractions of the salbutamol sulphate/chitosan systems were significantly greater than was observed for micronised salbutamol sulphate (Table 3). Respirable fractions for the co-spray dried powders were comparable with respirable fractions determined for the commercial Ventolin Rotacaps® formulation ($39.97 \pm 1.86\%$), the formulation of which contains inert coarse carrier particles (30–90 μm) included to aid flow and dispersibility of drug particles, which can be highly cohesive when micronised [38].

4. Conclusion

Spray drying from aqueous media containing HCl or acetic acid affected the morphology and degree of acetylation of chitosan, the presence of acetic acid resulting in increase acetylation of the polymer during processing.

There was no detectable difference in morphology between non-crosslinked and crosslinked co-spray dried composite particles. However, with increasing salbutamol loading particles became less spherical, taking on a collapsed appearance.

Spray dried chitosan–salbutamol sulphate microparticulates were amorphous.

Chitosan–salbutamol sulphate compressed discs showed delayed release of salbutamol sulphate. The general power law equation fitted the data better than the $t^{0.5}$, mono- or bi-exponential models and gave n indices greater than 0.5. Both crosslinked and non-crosslinked composites swelled during release, the former to a greater extent. The release data for crosslinked composites gave slightly higher n values than the corresponding non-crosslinked composites, consistent with the increased swelling of these systems. Crosslinking did not dramatically alter the drug release behaviour.

Drug release from the microparticulates was extremely rapid < 5 min. Twin impinger analysis demonstrated high respirable fractions for the co-spray dried microparticles, which may have potential for pulmonary delivery.

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