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Research Article

Uniform Chitosan Microparticles Prepared by a Novel Spray-Drying Technique

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Particle size and morphology are important properties of pharmaceutical particles. Preparation of microparticles with uniform particle size and morphology is necessary in order to systematically relate these properties to the release behavior and other functionalities such as drug encapsulation and dissolution. In this study, we successfully prepared monodisperse, nonagglomerated chitosan microparticles in a single step by a novel spray-drying technique. The control of particle size and morphology of spray-dried microparticles was investigated experimentally. Microparticles with larger particle size can be produced when chitosan precursor of higher concentration was used. Storage time of chitosan precursor, drying temperature, and addition of lactose were shown to be crucial parameters that affect the particle morphology. Appropriate choice of the drying temperature and precursor storage time permitted control of the particle morphology, ranging from nearly spherical to cap-shaped. Surface characteristics of the particles can be finely tuned by the amount of lactose added into the chitosan precursor.

1. Introduction

Encapsulation of therapeutic agents into polymeric particles has been widely used in drug delivery systems. Different methods have been used to prepare polymer microparticles, such as emulsification [1], ionic gelation [2], coacervation/precipitation [3], and spray drying [4]. Among these methods, spray drying is highly attractive due to the fast particle formation process and easily scale up. However, microparticles obtained from conventional spray dryers generally display nonuniform properties, such as different sizes and morphologies. As crucial parameters of pharmaceutical particles, particle size and morphology influence almost every aspect of particle functionalities like degradation, flowability, uptake, and clearance [5–7]. Naturally, fabrication of microparticles with uniform and controllable size and morphology is highly desirable and imperative.

Recently, a novel spray-drying technique has been introduced to prepare uniform microparticles [8, 9]. A specially designed microfluidic aerosol nozzle (MFAN) system, capable of atomizing the precursor solution into discrete droplets in a single stream, was used as the atomizer. These droplets were well dispersed and dried in a microfluidic-jet spray dryer (MFJSD).The drying process was specially designed to ensure identical drying experience for every individual droplet to acquire homogeneous microparticles.

In this study, we tackled the issue of preparing uniform microparticles using the novel spray-drying technique. Specific attention was paid to the control of particle size and morphology of spray-dried microparticles. Chitosan, the N-deacetylation products of chitin, was used as the model polymer. Chitosan has been employed for microencapsulation purposes since the 1980s due to its availability and excellent properties, such as being nontoxic, bioadhesive, and biodegradable [10]. Chitosan microparticles as drug carriers not only protect the drug molecules from degradation *in vivo*, but also improve their uptake and bioavailability [11]. These properties render chitosan microparticles potential carriers for protein, peptide, and vaccine.

By controlling the droplets formation process, chitosan precursors were atomized into droplets of similar droplet size. Chitosan precursors of different concentrations were

used to control the particle size of spray-dried microparticles. We speculated that drying conditions and viscosity of chitosan precursors would have somewhat effects on the particle morphologies due to their influences on the solvent evaporation rate or solute diffusion rate during spray-drying process [12]. The control of viscosity was realized by the change of chitosan concentration and controlled degradation reactions [13]. Different amounts of lactose were also added into the chitosan precursors to investigate the influence of small molecules on particle morphology of spray-dried microparticles.

2. Materials and Methods

2.1. Preparation of Chitosan Precursors. Chitosan precursors of different concentrations (0.5 wt%, 1.0 wt%, or 1.5 wt%) were prepared by dissolving certain amount of chitosan (low molecular weight, Sigma, Australia) into 1% (v/v) acetic acid aqueous solution. For the effect of lactose, different amount of lactose (α-lactose monohydrate, Sigma, Australia) was added into 1.5 wt% fresh chitosan solution to achieve the chitosan: lactose ratio of 18 : 1, 12 : 1, or 6 : 1 (w/w), respectively. Deionized water was used for precursor preparation (Millipore, Australia).

2.2. Degradation of Chitosan. The chitosan solutions were placed in screw capped glass bottles and stored in an incubator at 35°C. The acid degradation process was proceeded for 18 days.

2.3. Physicochemical Characterization. At certain time intervals, samples were taken for determination of viscosity, pH, conductivity, and FTIR-ATR spectrum. Viscosity of chitosan solutions was determined with a rotational viscometer at the rotation speed of 50 rpm (Visco Basic Plus, Fungilab, Spain).The measurements were conducted at 25°C, and the temperature was controlled by a water bath. FTIR-ATR spectrum was performed on a Perkin Elmer spectrometer equipped with an ATR (attenuated total reflectance) detector in the wavelength range of 4000–560 cm^{-1}, at a spectral resolution of 4 cm^{-1}.

2.4. Preparation of Uniform Microparticles. Monodisperse droplets of different precursors were generated by MFAN with the orifice diameter of 75 μm. The droplet breakup mode was observed by a digital SLR camera (Nikon, D90) with a speed-light (Nikon SB-400) and a microlens (AF Micro-Nikkor 60 mm f/2.8D). The piezoelectric frequency applied was adjusted to best achieve a monodisperse droplet formation [8]. These monodisperse droplets were dispersed and dried in MFJSD. In a typical experiment, inlet temperature of 180°C was used. Inlet temperatures of 160°C, 140°C, and 120°C were also set to investigate the influence of drying temperature on particle morphology.

2.5. Characterization of Microparticles. Images of chitosan microparticles were recorded by light microscopy (Motic B1-223A, UK) and scanning microscopy (SEM, JEOL JSM-840A, Japan). Particle size and size distribution were measured and analyzed using the software package Motic Images Plus 2.0 ML and ImageJ. A minimum of 500 microparticles was analyzed for each sample. The average particle size ($\overline{d}$) was defined as $\overline{d} = \sum_i^n d_i/N$, and the standard deviation of particle size was described as $SD = \sqrt{\sum (d_i - \overline{d})^2/(N-1)}$, where d_i is the diameter of each particle, N is the total number of particles counted.

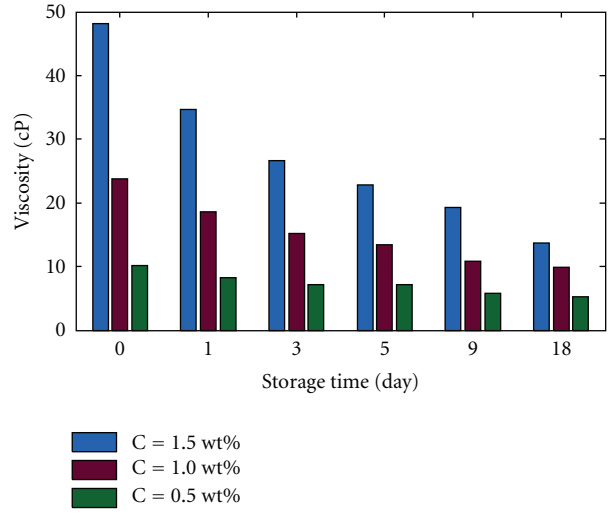


Figure 1: Viscosity of chitosan solutions at different storage time.

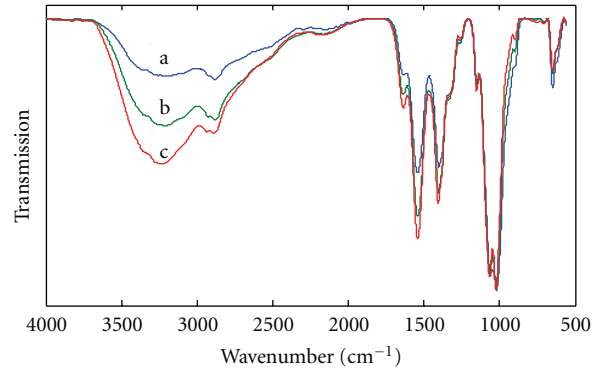


Figure 2: FTIR-ATR spectrum of 1 wt% chitosan solution at different storage time: (a), 0 day; (b), 1 day; (c), 18 days.

3. Results and Discussion

3.1. Physicochemical Properties of Chitosan Precursors. The viscosity of different chitosan solutions was presented in Figure 1. The viscosity of chitosan solution increased with the concentration, which might be attributed to increasing intermolecular entanglement leading to more restriction in the freedom of movement of individual chains [14]. For each concentration, the change in viscosity was noticeable during storage time. The viscosity decreased with increased aging time which was consistent with previous studies in [15]. The decline of viscosity was the result of polymer

Table 1: pH and conductivity of chitosan solutions at different storage times.

Storage time (day)	pH			Conductivity (mS/cm)		
	0.5 wt%	1.0 wt%	1.5 wt%	0.5 wt%	1.0 wt%	1.5 wt%
0	2.99	3.44	3.69	1.11	1.74	2.25
3	3.02	3.43	3.62	1.11	1.75	2.27
6	2.96	3.31	3.52	1.12	1.74	2.24
9	3.05	3.45	3.66	1.11	1.75	2.26
18	3.10	3.44	3.69	1.12	1.75	2.27

degradation related to the decrease of molecular weight of chitosan [15]. A higher reduction of viscosity was observed for high concentration solution. The viscosity of 0.5 wt%, 1.0 wt%, and 1.5 wt% chitosan solutions decreased by approximately 48%, 58%, and 72%, respectively, after 18 days.

From Table 1, the pH value and conductivity of chitosan solution increased with higher chitosan concentration. As the concentration of chitosan increased, an increase amount of H^+ was consumed by protonation of the free amino groups in chitosan molecules causing the pH value to increase. However, the pH and conductivity of chitosan solution exhibited similar properties during storage. The practically unchanged free acid concentration in the hydrolysis process has also been proven elsewhere by conductimetric titration [16]. Meanwhile, the activation energy for equivalent conductivity of chitosan solutions was found to be virtually independent of the molecular weight [17].

FTIR-ATR was also applied to examine the chemical and structural changes of chitosan in the precursors. FTIR-ATR had been shown to be a useful and nondestructive method for detecting changes of particular functional groups in polymer molecules [18]. Figure 2 showed the FTIR-ATR spectrum of chitosan at different storage time. The ATR-IR spectrum determined was basically unchanged for chitosan at different storage time, except for the relative intensities of some peaks. The acid hydrolysis of chitosan was a combination of the hydrolysis of the O-glycosidic linkage and the N-acetyl linkage [19]. The two processes would bring –OH or $–NH_2$ to chitosan molecules, which induced an increase of intensity in related peaks.

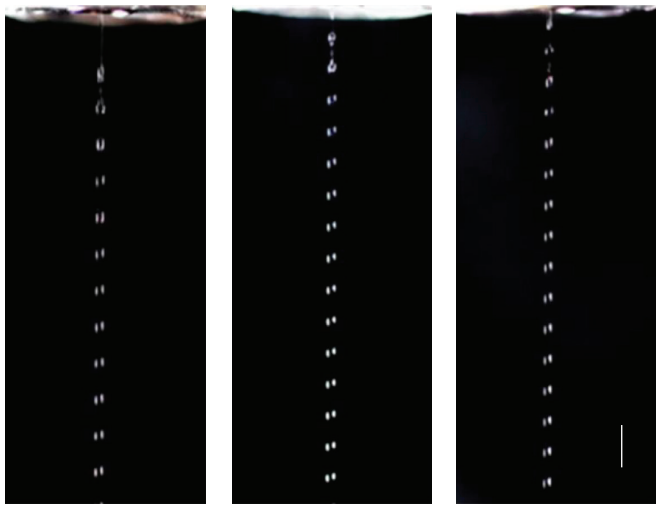
3.2. Spray Drying of Uniform Microparticles

3.2.1. Formation of Monodisperse Droplets. Figure 3 showed the photographs of uniform and monodisperse droplets generated by MFAN. The uniformity of initial droplet was the prerequisite of uniform spray dried microparticles. However, a narrow size distribution could in theory be achieved only if the initial droplet size was well controlled, with no breakage or coalescence of the droplets occurring in the process [20]. The good dispersion of initial droplets was shown in Figure 4.

3.2.2. Effect of Chitosan Concentration. Chitosan solutions of different concentrations were spray dried under the same drying conditions (inlet temperature 120°C).The SEM photographs and size distribution in Figure 5 showed that chitosan microparticles of great uniformity were obtained successfully. The mean diameter of microparticles obtained from 0.5, 1.0, and 1.5 wt% chitosan solutions were 35.1 ± 2.22, 49.2 ± 2.42, and $56.2 \pm 4.59\,\mu$m, respectively. Therefore, microparticles with a required size can be easily prepared with this method by adjusting the chitosan concentration in the precursor solution. From Figure 1, it is clear that the viscosity of chitosan solution was greatly dependent on chitosan concentration. However, the difference in viscosity did not translate into any visible effect on particle morphology. At the initial drying stage, the quick evaporation of water concentrated the solution quickly, so that the droplets produced from precursors in the current concentration range followed a similar drying process to produce the same morphology. Chitosan solutions of different concentrations after a storage time of 18 days were also spray-dried, again with no noticeable correlation between concentration and the morphology of microparticles (Figure 6).

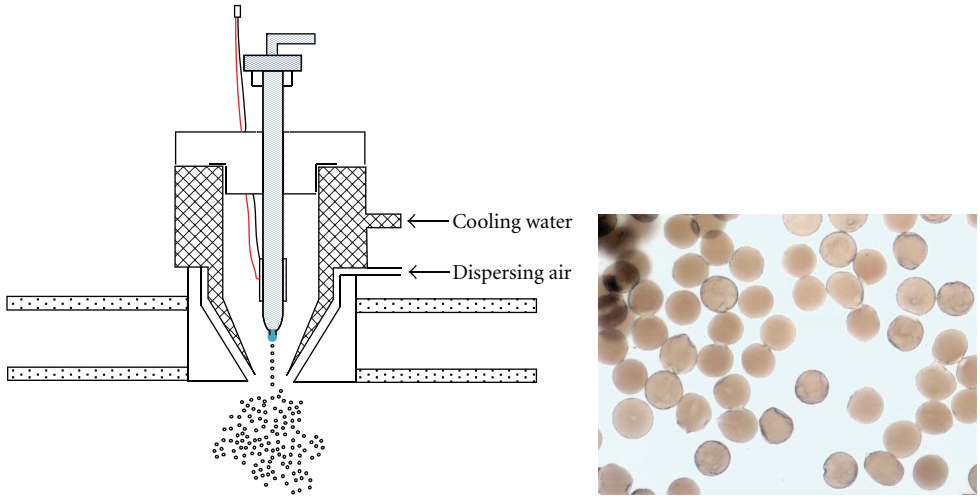
3.2.3. Effect of Drying Temperature. In order to investigate the effect of drying temperature on the morphology of spray-dried chitosan microparticles, four inlet temperatures (180°C, 160°C, 140°C, and 120°C) were used. The outlet temperatures were 100°C, 98°C, 94°C, and 89°C corresponding to the inlet temperatures. Figure 7 showed the SEM photographs of chitosan microparticles obtained at different drying temperatures. It was found that the drying temperature played an important role in the morphology of spray-dried chitosan microparticles. Significant deformation of chitosan microparticles under high drying temperature resulted in cap-shaped microparticles (Figures 7(c) and 7(d)), while, microparticles obtained from low drying temperature were nearly spherical (Figures 7(a) and 7(b)). This phenomenon can be explained by the different drying rate at different temperature. High inlet temperature resulted in a high drying rate, quickly forming a dry crust. When the internal vapour pressure exceeded the range of pressure that the crust could withstand, the semidried droplets collapsed and formed dimples [21]. In addition, a shorter drying time resulted in more extreme drying behavior (i.e., quicker heat and mass transfer, shorter drying time, more violent drying process), thus the tendency to deform increased [22].

3.2.4. Effect of Storage Time. Chitosan solutions at different storage time were spray-dried under the same drying conditions (inlet temperature 120°C). The SEM photographs of obtained chitosan microparticles were shown in Figure 8, and storage time was shown to have a great influence on particle morphology. Fresh chitosan solution resulted in nearly spherical particles. With increasing storage time, the dimples in microparticles became bigger, and the morphologies changed into cap-shaped. Although the viscosity of chitosan solution significantly changed during storage, the apparent viscosity change alone might not be enough to explain the considerable differences in particle morphology. As from previous discussion on the influence of chitosan



(a) (b) (c)

Figure 3: Typical photographs of monodisperse droplets formation: (a) 0.5 wt%; (b) 1.0 wt%; (c) 1.5 wt% (storage time: 0 day). Scale bar: 5 mm.



(a) (b)

Figure 4: (a) Schematic diagram of droplets dispersion. (b) Droplets of chitosan solution dispersed in sodium citrate solution.

concentration, we found that the morphologies of microparticles prepared from fresh chitosan solution of different viscosities were relatively similar. Therefore, the morphology change was more likely to be ascribed to the change in the molecular weight of the chitosan, as indicated by the change in the apparent viscosity [15]. Under the same mass content and drying temperature, droplets from each solution would undergo similar drying process. With increasing storage time, the molecular weight of chitosan decreased. As a result, the dry crust formed would be of lower strength that could easily collapse during subsequent drying process, leading to cap-shaped particles. For the spray drying of PLA and PLGA microparticles, it was also reported that the polymer molecular weight was a more important factor than the apparent viscosity of solution for the morphology of spray-dried microparticles [23]. Thus, special attention needs to be paid to the variability of chitosan molecules which might cause irreproducibility of chitosan based materials.

3.2.5. Effect of Lactose. The viscosity of solution with chitosan lactose ratio of 6 : 1, 12 : 1, 18 : 1 was 44.13, 43.77, and 42.55 cP, respectively. Addition of lactose only slightly increased the viscosity of chitosan solutions compared with pure 1.5 wt% chitosan solution (42.37 cP). These solutions were spray-dried under the same drying condition (inlet temperature 120°C), and the SEM photographs of obtained microparticles were shown in Figure 9. Lactose effectively imparted spherical shape and surface smoothness to the microparticles. As expected, when more lactose is added, the surface smoothness and sphericity become better. The following explanation was suggested. Lactose is disaccharide

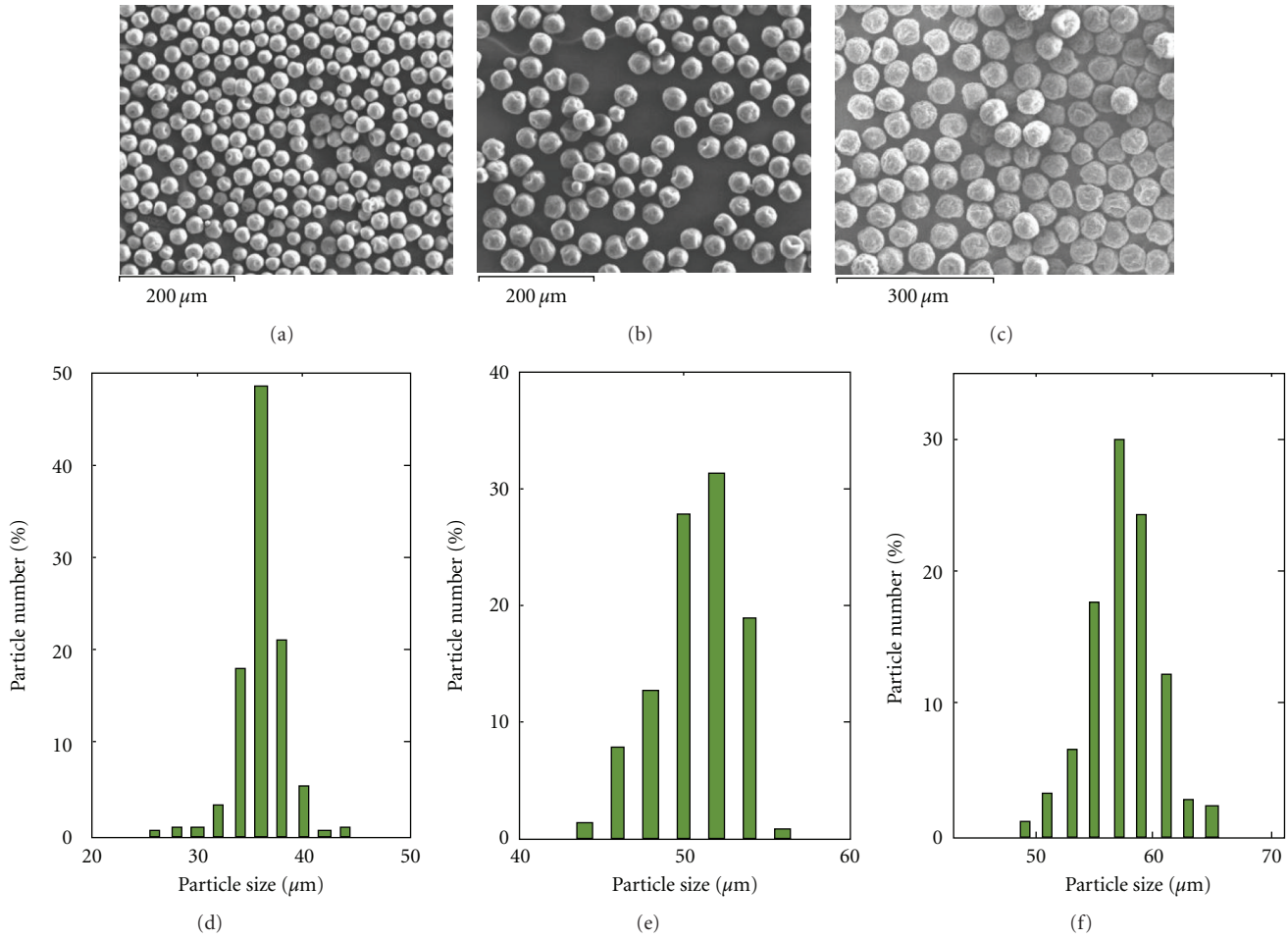


Figure 5: SEM photographs and size distribution of chitosan microparticles prepared with different chitosan concentrations: (a), (d) 0.5 wt%; (b), (e) 1.0 wt%; (c), (f) 1.5 wt% (storage time: 0 day).

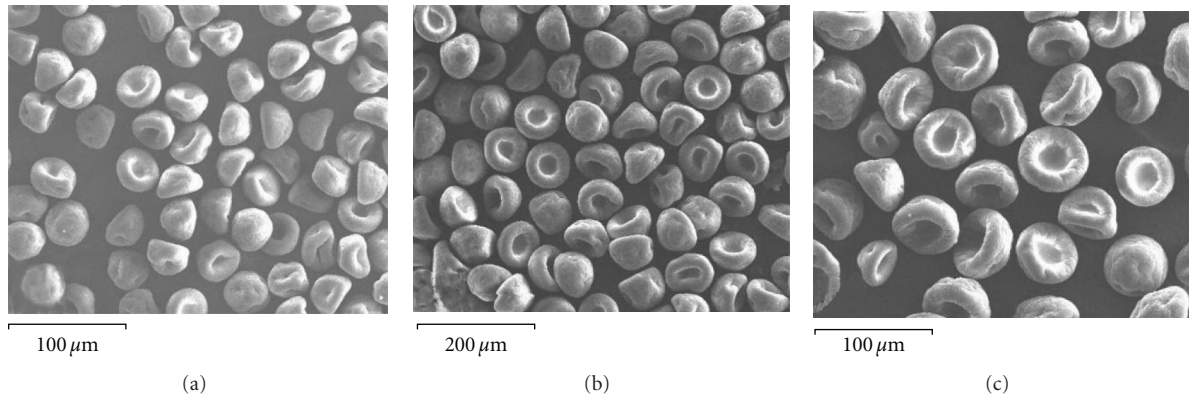


Figure 6: SEM photographs of chitosan microparticles prepared with different chitosan concentrations: (a) 0.5 wt%; (b) 1.0 wt%; (c) 1.5 wt% (storage time: 18 days).

which has much lower molecular weight and shorter molecular chain than chitosan. Lactose was more mobile during the water evaporation process, and it can take the place of water to some extent and reduce the shrinkage [24]. Besides, the increasing solid content in the droplets also helps to reduce particles distortion and surface folders [22, 25].

4. Conclusion

This paper investigated the influence of formulation variables on the size and morphology of spray-dried chitosan microparticles. Particles with uniform size and morphology were successfully prepared by a novel microfluidic jet spray

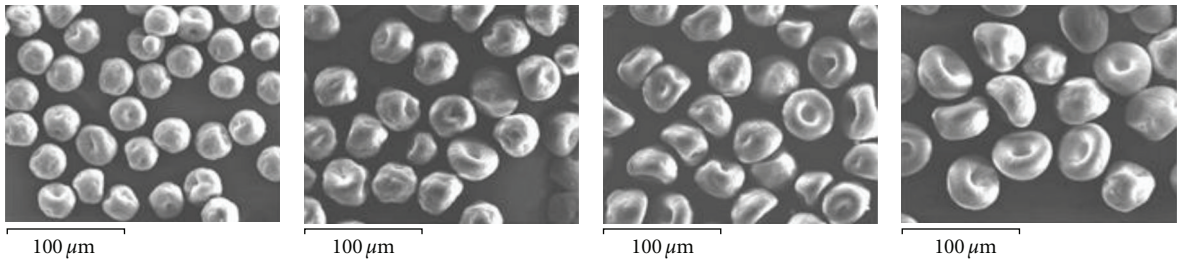


Figure 7: SEM photographs of chitosan microparticles spray dried under different inlet temperatures: (a)120°C, (b)140°C, (c) 160°C, and (d)180°C (1.0 wt% chitosan solution, storage time: 0 day).

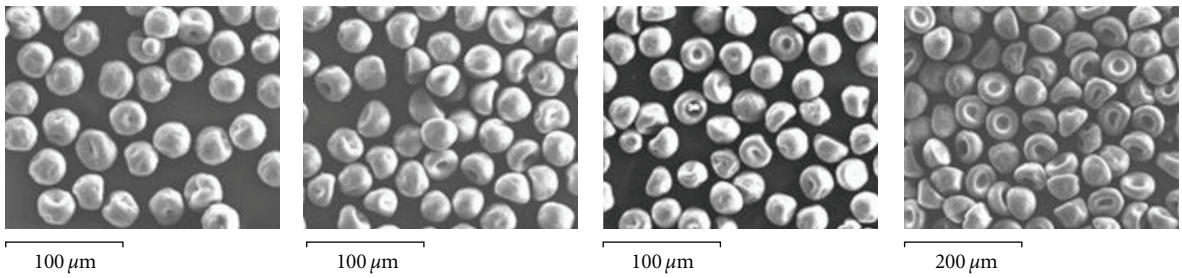


Figure 8: SEM photos of chitosan microparticles spray dried at different storage times: (a) 0 day, (b) 1 day, (c) 3 days, and (d) 18 days (1.0 wt% chitosan solution, inlet temperature 120°C).

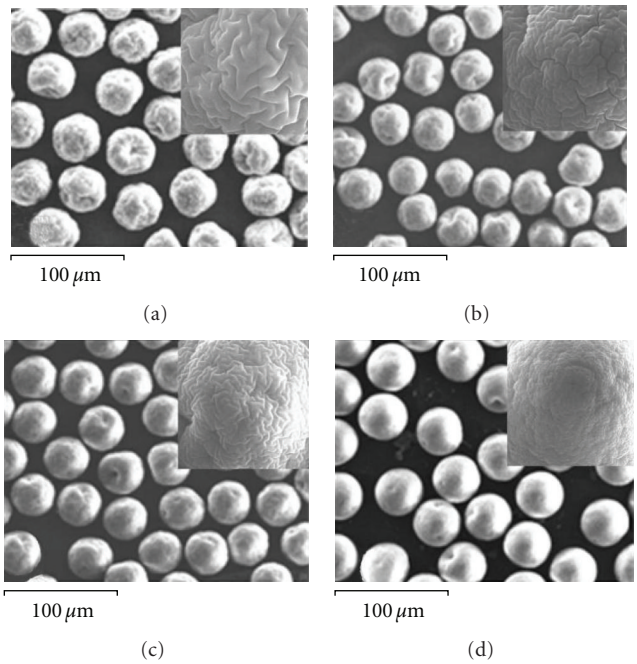


Figure 9: SEM photographs microparticles prepared with solutions of different chitosan lactose ratio (w/w): (a) no lactose, (b) 18:1, (c) 12:1, and (d) 6 : 1 (chitosan concentration 1.5%, storage time 0 day).

drier developed at Monash University. To the best of our knowledge, the preparation of uniform chitosan microparticles by spray drying has never been reported before. Here, the control of particle size could be directly done by adjusting the concentration of chitosan solution in the precursor. In particular, we have demonstrated that drying temperature, storage time of chitosan solution, and lactose addition played a key role in determining both the particle shape and surface characteristic. Thus, it would be feasible to prepare chitosan microparticles of certain size and morphology by selecting the proper drying condition and composition of chitosan solution. This capability provides the potential to generate chitosan microparticles with specific properties for applications including targeted drug release.

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