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On the formation of “coral-like” spherical α-glycine crystalline particles

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ABSTRACT: Spray drying can be used to form crystalline amino acid particles of specific size and morphology by adjusting the process conditions. Combined with post-drying humidity treatment, porous and spherical α-glycine particles with “coral-like” morphology were produced by spray drying. Glycine solutions of 8 wt% and 18 wt% were spray dried at inlet temperatures between 105 °C to 185 °C using a microfluidic jet spray dryer. The inlet drying temperatures had
no significant effect on the polymorphic forms of glycine, but significantly affected their morphology in terms of surface roughness and internal structure. Spray drying provides an easy fabrication approach of spherical α-glycine particles of the particle size in the range of 70-100 μm with less separation, which is a significant advantage over the multiple-step cooling or evaporation processes.

Keywords: spray drying; α-glycine; spherical crystalline particles; humidity treatment; porous structure
1. INTRODUCTION

The importance of particle morphology is well-known as it is directly related to the manufacturability and bioavailability of the powder [1]. Sphericity of particles can improve flowability and de-agglomeration, resulting in the ease of manufacturability [2]. The small size of individual crystalline particles can enhance bioavailability due to the ease of dissolution [3]. Glycine, the simplest amino acid, is widely used as a model to investigate the manipulation of crystalline particle size [4, 5]. Common ways of producing crystalline particles of glycine via cooling or evaporation crystallization in a large batch crystallizer normally result in polydisperse particles with sizes ranging from tens to several hundreds of micrometers [6]. Glycine particles produced in these ways are usually needle-like, bipyramidal-shaped [7] or plate-like [8]. The wide range of size and morphology limits the utility and the overall applicability of the end products. Researchers have thus endeavored to develop ways to effectively control size and morphology of glycine particles in a semibatch [9] or batch process [5, 10].

Spray drying can be used to produce glycine particles from aqueous solution. Some degree of control over particle size and morphology can be achieved by adjusting the spray drying conditions [11]. Among the conditions, the dehydration rate is an essential factor mainly controlled by the drying temperatures and the precursor feed concentrations. The overall time to produce glycine particles via spray drying is in the order of seconds, as compared to several hours in batch crystallization processes [4]. Spray drying provides an easy fabrication of glycine particles in a one-step process, as compared to multiple steps including seeding, filtration and drying in a batch crystallization. Previously, the morphology and size of spray-dried glycine particles were shown to be influenced by the effect of the precursor pH [12]. However, the effects of dehydration rate on the morphology of spray-dried glycine particles are still unclear.
Herein, we utilized a microfluidic drying technique to investigate how the dehydration rate affects the particle shrinkage, morphology and polymorphism of glycine particles. The spherical α-glycine particles of the size in the range of 70-100 μm with various morphologies were produced at various process conditions. Furthermore, we found that spherical “coral-like” glycine crystalline particles consisting of fine α-glycine crystal grains could be produced by combining the spray drying process with post-drying humidity treatment. The possible mechanisms for the formation of “coral-like” α-glycine particles were proposed.

2. EXPERIMENTAL DETAILS

2.1 Fabrication of spray-dried microparticles. Glycine particles were fabricated by spray drying using a microfluidic jet spray dryer [13, 14] to produce monodisperse particles. The original glycine (≥99 %, G7126-500G, Sigma-Aldrich, Australia) confirmed as α-glycine (Fig. S1) was used without further purification. The aqueous solution with 8 wt% and 18 wt% glycine concentrations of pH between 6.2 - 6.3 were used as precursors. The concentration at 18 wt% was approaching the saturated concentration of 18.36 wt% at 20 °C and 20.00 wt% at 25 °C [15]. Glycine solutions were spray dried at different inlet drying temperatures of 105, 145 and 185 °C. To monitor the generation of monodisperse droplets, a digital SLR camera (Nikon, D90) with a speed light and a microlens was used to take images of generated droplets. The details of solution preparation and the spray drying process were described in the Supporting Information.

2.2 Post treatment of spray-dried glycine particles. Small amounts of the spray-dried glycine samples were placed in a petri dish to form a thin layer. The petri dish was stored in two different conditions, a humid condition (~76 % RH) and in a dry air condition (< 20% RH) at room temperature (20 ± 1 °C), respectively. The humid condition was created using a saturated
sodium chloride (102406, Merck KGaA, Germany) solution in the sealed desiccator, whereas the
dry air condition storage was created in the desiccator with self-indicating blue silica gel beads
(ST043, Chem-supply Pty LTD, Australia).

2.3 Characterization of spray-dried and the post-drying treatment glycine particles.

Particle morphology. Glycine particles were fixed to an aluminium sample stub using a
conducting carbon tape and then sputter-coated with ~3 nm gold-palladium to produce a
conductive surface for Scanning Electron Microscope (SEM) observation. Nova NanoSEM (FEI
450 FEG) with an accelerating voltage of 2 kv or 3kv with the spot size 2-3 and a working
distance of ~ 4.8-5.2 mm was used.

Determination of polymorphic form(s). The samples were analyzed by Philips 1130 (Philips,
The Netherlands) X-ray powder diffractometry (XRD) within 30 minutes after spray drying.
XRD pattern were obtained with Cu Kα radiation (λ=1.5405 Å) at 40kv and 25 mA. The
deliberately crushed glycine particles were placed on a single crystal quartz specimen substrate
for XRD analysis. Each sample was scanned between 10 and 50 ° in 20 at the scanning rate of 2
°/min⁻¹ with a step size of 0.02 °C.

2.4 Shrinkage behavior of spray-dried particle. The initial droplet size and the final particle
size were defined as the initial diameter of the droplet (d₀) and the final diameter of the dried
particle (d). The parameters d₀ and d were obtained using the Image J software (freeware),
based on images taken during the droplet generation and SEM images of glycine particles. Feret
diameter, the longest distance between any two points along the boundary of the particles, was
used as the equivalent particle diameter. The average diameter was reported as the initial droplet
or final particle diameter. It is noted that a small systematic error may be also presented due to the preferential orientation of particles. The average particle size ($\bar{d}$) was defined as

$$\bar{d} = \frac{1}{N} \sum_{i=1}^{N} d_i = \frac{d_1 + d_2 + \ldots + d_N}{N}$$  \hspace{1cm} (1)

The standard deviation of the particle size was described as

$$SD = \sqrt{\left(\sum_{i=1}^{N} (d_i - \bar{d})^2 \right)/(N-1)}$$  \hspace{1cm} (2)

where $d_i$ was the diameter of the $i^{th}$ particle and $N$ was the total number of particles counted from SEM images. To minimize the errors, $N \geq 40$ particles were measured.

To investigate the drying process, the deviation between the perfect shrinkage and the shrinkage ratio ($K_{sh}$) was calculated. Herein, $K_{sh}$ (%), shrinkage ratio is defined as

$$K_{sh} = \left(\frac{d}{d_0}\right) \times 100$$  \hspace{1cm} (3)

The perfect shrinkage was calculated according to Lin et al [16]. The perfect shrinkage theory is based on the assumption that the change in droplet diameter is solely dependent on moisture removal from droplet without any crust formation.

3. RESULTS

3.1 Effects of drying temperatures and feed concentrations

**Drying behaviors.** Powders from 8 wt% glycine solution at different drying temperatures showed similar moisture contents of around 2.5 % (Table 1). By contrast, the moisture contents of the spray-dried particles from the 18 wt% solution were all below 1.5 %; at the drying temperature of 185 °C, the moisture content even decreased to 0.5 %. The relatively low moisture contents in the spray-dried glycine powder are due to the highly crystalline form of glycine particles with less possibility of absorbing moisture. The sizes of the generated droplets
in MFJSD were in the range of 150-180 μm and the corresponding sizes of the spray-dried particles were in the range of 70-100 μm with relatively small standard deviations.

It was found that the solution concentration affected the shrinkage ratio more significantly than the drying temperature. When the glycine concentration was increased from 8 to 18 wt%, the shrinkage ratio at 105 °C increased from 42.3 ± 2.4 % to 56.7 ± 4.6 %. There will have a higher propensity for the 18 wt% solution to reach the saturated concentration (18.3 wt% at 20 °C) than the 8 wt% glycine solution, causing an early precipitation of glycine on the surface [17]. Thus, an early precipitation of glycine on the surface would have resulted in a larger particle size and thus a higher shrinkage ratio. On the other hand, the shrinkage ratio only increased slightly from 42 % to 48% for the 8 wt% glycine within temperature range investigated (Fig. 1). A similar trend was found for the 18 wt% solution.

The deviation from perfect shrinkage across all drying temperatures was almost identical between the two feed concentrations (Fig. 1). The perfect shrinkage ratio is based on the assumption that a given droplet shrinks isotropically as the moisture evaporates, resulting in a solid and dense particle. The extent of the deviation is an indication of the porosity of the spray-dried particles.

**Particle morphology.** The original commercially available glycine crystals are typically large bipyramidal-shaped particles with size in the range of 450-600 μm, whereas the spray-dried glycine particles are small spherical particles with size in the range of 70-100 μm (Fig. 2). Spray-dried particles displayed generally spherical shapes for all samples (Fig. 3). The particles of spherical shape were reported to have better flowability than those of cubic or plate-like shape due to the relative smaller particle contact area [2]. In our work, the bipyramidal-shaped glycine
transformed into spherical particles via spray drying, suggesting that spray drying can be used as a practical technique to manipulate the shape of crystalline materials for better flowability. It should be taken into account that other parameters, such as the moisture content, the electric charge and the density of the particles, will also affect the flowability [18]; however, the investigation of the influence of those parameters on the particle flowability are beyond the scope of this work. Hence, the direct comparison of the flowability between the original glycine and spray-dried glycine will not be included in this work. Nevertheless, this work is a first step towards manipulating the flowability by controlling the crystal shape.

Microparticles obtained from the different feed concentrations (8 wt% and 18 wt%) displayed similar morphologies. Those obtained at different drying temperatures, however, presented noticeable differences in the particle morphology. Specifically, the particles dried at 185 °C showed a dimple-like shape with a packed surface, whereas those with sheet-like surface were observed at 105 °C. Hollow structures and large block-shaped crystals constituting the crust were found for particles dried at 185 °C, in contrast to dense particles found at 105 °C. The morphology obtained from the intermediate temperature (145 °C) presented combined features of the two types of particles. These findings suggest that the feed concentrations have no significant effect on particle morphology in terms of surface roughness and internal structure. However, these particle morphologies were significantly affected by the drying temperatures.

**Glycine crystallinity and polymorphism.**

The spray-dried glycine particles were crystalline with sharp peaks identified from their XRD patterns (Fig. 4), in good agreement with previous reports on spray-dried glycine [12]. Previously, the pH was reported to affect the dimeric growth unit of α-glycine, making α-glycine the preferred polymorph, as α-glycine crystallizes from aqueous solution at close to the
isoelectric point (pI 5.97) [19]. The pH of the precursor solution used in the current experiments was around 6.2 - 6.3. As expected, the spray-dried glycine samples were predominantly in the α-form, rather than β- or γ-form. The spray-dried glycine was further confirmed to be α-glycine by FTIR (Fig. S2).

It is noteworthy that the original glycine material was mainly α-glycine and thus the spray-dried particles remained as the stable form of α-glycine. Thus spray drying in this case can be employed to change the shape and size of the original glycine powder without any significant loss in either the particle crystallinity or the polymorphic stability.

3.2 Effects of post drying treatment

Significant differences in morphology between the humidity-treated particles post drying and the freshly spray-dried particles can be observed in Fig. 5. “Coral-like” spherical particles was found in all samples dried from the high feed concentration (18 wt%) at various temperatures (Fig. 5, A-C). The spherical porous particles consisted of crystal grains. At the cross section of the particles, the crystal grains could be clearly noticed in the internal section of particles (Fig. 5 A1, B1, C1). For the glycine particles dried at 185 °C, “randomly” packed block crystal grains were observed throughout the whole structure of the particle, in contrast to the relatively hollow structure of particles observed from the freshly spray-dried samples.

Similar changes in morphology can be found in the particles dried from the 8 wt% solution. The cracks between the crystals on the particle surface could be clearly observed. However, unlike samples dried from the high feed concentration solution, the initial framework observed from the freshly spray-dried particles was still visible in the samples from the low feed concentration solution exposed to humidity (Fig. 5 D1, E1 & F1). The morphology of the
samples dried from the 8 wt% solution exposed to humidity treatment for a month (Fig. S3 D) showed mostly similar features to those dried from the 18 wt% solution stored for one week (Fig. 5 A). Despite significant changes in morphology, the polymorphic form of all spray-dried particles in humidity treatment was still in the α-form, as confirmed by XRD diffractions (Fig. S4). This polymorphism stability of “coral-like” glycine is a significant advantage over the glycine crystals produced from cooling crystallization, from which the α-glycine obtained was reported to undergo polymorphic changes during humidity storage [20].

4. DISCUSSIONS

4.1 Mechanism(s) leading to particle morphologies at different drying temperatures

The different morphologies of the spray-dried glycine at various temperatures can be explained by the simultaneous effect of the drying and crystallization processes occurring during spray drying [21]. When the generated droplet enters the drying chamber, water removal takes place on the droplet surface, which induces the water within the droplet to diffuse towards the droplet surface. Subsequently, when the internal diffusion could not replenish the surface moisture, the surface concentration will increase and progressively approach supersaturation. The supersaturation further induces the nucleation of glycine crystallites on the droplet surface, forming the basic spherical framework observed. The ultrafine crystallite on the surface was not detectable under the current resolution of the SEM technique. The presence of ultrafine crystallites constituting the particles on the surface was reported in crystalline mannitol particles produced via spray drying [22].

For a high drying temperature, the formation of hollow particles with relatively large internal crystals is caused by the comparably high level of evaporation rate. Once the surface framework is formed, the nucleation induces the decrease of concentration on the droplet surface due to
solid precipitation; however the evaporation rate is sufficient to maintain the supersaturation. Driven by the fast evaporation, the internal water transfers from the droplet onto the surface and thus a bubble is formed in the center, leaving to a hollow structure (Fig. 5. A3 & D3). The formation of the particle on the surface is rapid so that some liquid is trapped inside the particle and the evaporation rate of the solution is slowed down. Consequently, the increase of supersaturation of the trapped liquid is not considered as fast as that on the surface. The crystal growth is thus expected to be undertaken in a slow crystallization process leading to large crystalline grains internally.

For a lower temperature of 105 °C, the decrease in evaporation rate on the glycine particle results in the relatively dense particle structures. Once the surface framework is formed, the relatively slow water evaporation gradually assists the crystal growth of the existing nuclei, followed by the final precipitation of glycine solid in the centre. This led to a relative dense structure (Fig. 3 C3 & F3), which was also reflected in the less extent of deviation from perfect shrinkage (Fig. 1). The combination of these two scenarios occurred in the samples dried at the intermediate drying temperature, resulting in combined morphological features of particles dried at lower and higher temperatures. The schematic diagram (Fig. 6 I ) illustrates these mechanisms.

4.2 Mechanism(s) for the formation of “coral-like” glycine particles

The morphological changes leading to the “coral-like” particles may be attributed to the initial dissolution of glycine crystalline grains at the bridges between the crystals, followed by further recrystallization of the glycine solute during humidity treatment (Fig. 6 II). Glycine is highly soluble in water with the solubility of 27.6 g/100 g water at the temperature of 25 °C [15]. With
exposure to high humidity, the very fine crystals on the particle surface could partially dissolve due to the condensing water. The dissolved glycine may recrystallize on the existing crystals, resulting in the larger crystals. The surface energy theory on crystal growth suggests that the growing crystal would ensure that the crystal has a minimum surface free energy for a given volume [23]. The free energy is proportionally related to the crystal area, and therefore small crystal grains tend to grow into larger ones. With the ultrafine crystalline grains forming larger crystal grains, the particles were loosely formed by larger crystal grains with more cracks observed between individual crystal grains. Meanwhile, the dissolved glycine could flow internally due to the possible capillary force exerted on the condensing water. The internal structure thus changed from a hollow structure (obtained via spray drying) to a porous structure (obtained via post-drying humidity treatment) without any mass loss or increase. The mechanism is further confirmed from observing the samples dried at 185 °C exposed to humidity for a longer period (Fig. 7). As the humidity treatment is prolonged, large crystal grains with cracks on the surface were observed more clearly.

These findings suggest that humidity is an essential factor to tailor the surface morphology of the spray-dried glycine particles. Under high humidity, the spray-dried glycine particles could change into a more porous structure without any changes in the polymorphic form due to the partial dissolution and the subsequent recrystallization of α-glycine. Thus the post-drying treatment can be used to produce porous yet crystalline glycine particles. On the other hand, the spray-dried glycine samples are stable in terms of polymorphism and morphology when stored in dry air environment (Fig. S5).

Glycine particles with coral-like morphology completely dissolved in much less time compared to the original glycine samples or the spray-dried glycine particles of a similar Feret
diameter range from 140 to 474 μm. This dissolution observation was illustrated in Fig. 6 when the glycine particles were dissolved in a water droplet. Further analyses of samples stored in high humidity for up to two months showed that the particles were still in the form of α-glycine, implying the storage time could possibly be extended longer while maintaining the stable polymorphic form (Fig. S7).

CONCLUSIONS

Under the range of conditions studied, spherical crystalline α-glycine particles were obtained via spray drying. Drying temperatures had significant effects on the particle morphology due to the different drying and crystallization processes experienced by the particles. High drying temperature led to the formation of hollow particles, whereas low drying temperature led to the formation of relatively dense particles. The feed concentration had no significant influence on the glycine particle morphology. More importantly, porous “coral-like” spherical α-glycine particles consisting of fine crystalline grains were produced from spray drying combined with post-drying humidity treatment. The possible mechanisms for the formation of these coral-like particles can be attributed to the partial dissolution and the subsequent recrystallization of α-glycine. These particles might have potential for applications in drug delivery due to their spherical and coral-like morphology with polymorphic stability.

Supporting Information

Extended details of the experiments and auxiliary results related to the present study are available as supplementary data.

ACKNOWLEDGMENT
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REFERENCES
Table 1. Properties of spray-dried glycine particles from different feed concentrations at drying temperatures of 105, 145, and 185 °C.

<table>
<thead>
<tr>
<th>Drying temperature</th>
<th>105 °C</th>
<th>145 °C</th>
<th>185 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample name</strong></td>
<td>8 wt % 105</td>
<td>18 wt % 105</td>
<td>8 wt % 145</td>
</tr>
<tr>
<td><strong>Concentration</strong></td>
<td>8 wt %</td>
<td>18 wt %</td>
<td>8 wt %</td>
</tr>
<tr>
<td><strong>Moisture content ( %)</strong></td>
<td>2.2 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td><strong>Droplet size (μm)</strong></td>
<td>181.2 ± 27.7</td>
<td>181.6 ± 23.9</td>
<td>183.7 ± 22.7</td>
</tr>
<tr>
<td><strong>Particles size (μm)</strong></td>
<td>76.5 ± 7.9</td>
<td>103 ± 6.6</td>
<td>82.6 ± 12.4</td>
</tr>
</tbody>
</table>
**Fig. 1** The deviations from perfect shrinkage for glycine particles spray dried at different temperatures and concentrations.

**Fig. 2** Microscopic images of (a) original glycine crystals; and (b) spray-dried glycine particles.
Fig. 3 SEM images of the overview of spray-dried glycine microspheres (A1-F1) with that of an individual particle (A2-F2) and a crushed particle (A3-F3). Glycine microspheres with 18 wt% spray dried at 105, 145 and 185 °C are labeled as A-C series; 8 wt% spray dried at 105, 145 and 185 °C are labeled as D-F series.
**Fig. 4** XRD pattern of glycine particles spray dried from 8wt% and 18 wt% at 105, 145, and 185 °C. The standard XRD patterns of α-glycine (PDF 32-1702), β-glycine (PDF 02-0171) and γ-glycine (PDF 06-0230) were extracted by JADE software (MDI JADE 6 Materials Data Pattern Processing).
Fig. 5 SEM images of an individual particle (A1-F1) and a crushed particle (A2-F2). SEM images of the overview of humidity-treated glycine particles (inserted in A1-F1). Specifically, glycine microspheres obtained from 18 wt% solution spray dried at 105, 145 and 185 °C, followed by humidity treatment in 76% RH for one week post spray drying, are labeled as A-C series; obtained from 8 wt% solutions, labeled as D-F series. The unlabeled scale bar is 100 μm and the white arrows illustrate the presence of cracks between crystals.
Fig. 6 Schematic illustrations demonstrate the formation of spherical glycine particles at various temperatures (I) and the “coral-like” particles under humidity treatment (II).
**Fig. 7** Demonstration of the crystal growth process of one typical spray-dried glycine particles stored in around 76% relative humidity at various periods. The individual particle is on the top and a crushed one is at the bottom.

8 wt% 185 °C samples stored in 76 % RH at various time
Graphical Abstract

Original glycine

Spherical α-Glycine micro-particles

“Coral-like” α-Glycine aggregates

Particle size (μm)

Spray drying

High humidity treatment

This image has been digitally colourred to highlight the coral-like shape.
Highlights

- We demonstrated the fabrication of spherical “coral-like” α-glycine particles.
- Spherical α-glycine particles of 70-100 μm were fabricated via spray drying.
- The drying temperatures had significant effects on internal structure of particle.
- The “coral-like” particle morphology was induced by post-drying humidity treatment.
- The possible mechanisms for the formation of coral-like particles were proposed.