
Note

Size Reduction Efficiency of Alpha-Mangostin Suspension Using High-Pressure Homogenization

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Summary

In this study, we aimed to investigate the effects of stabilizers and processing parameters on the size reduction of alpha-mangostin (AMG) using high-pressure homogenization (HPH). The solubility of AMG in various stabilizers was studied. Selected stabilizers were used to prepare AMG suspensions by HPH under different conditions. After HPH, the particle size of AMG suspensions with stabilizers significantly decreased to microns. Percent size reduction efficiency of all AMG suspensions with each stabilizer increased with the increase in the number of homogenization cycles. Sodium lauryl sulfate and poloxamer188 provided a greater extent of particle size reduction than polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer. AMG suspensions with binary stabilizers at higher pressure were also prepared. The use of high pressure increased percent size reduction efficiency.

Key words: alpha-mangostin; size reduction; high-pressure homogenization
Over 40% of new candidate drugs are poorly water soluble.\textsuperscript{1) The low aqueous solubility results in poor absorption and bioavailability. To overcome this problem, the classical formulation approach for poorly water-soluble drugs is particle size reduction from coarse drug powder to ultrafine powder (micron/nanosized order). For the biopharmaceutical classification system (BCS) class II drugs having low solubility with good permeability, surface area enlargement of the drug powder can increase dissolution rate\textsuperscript{2)}, e.g. micronized griseofluvin\textsuperscript{3)} and nanosized cefpodoxime proxetil.\textsuperscript{4)} Milling is a process used to decrease drug size.\textsuperscript{5,6)} It can be classified into dry milling and wet milling, where wet milling provides smaller particles.\textsuperscript{6)} Wet milling can be further divided into milling with media, such as balls and beads, and milling without media, i.e. high-pressure homogenization (HPH). Media-less milling can avoid contamination from the abrasion of milling media.\textsuperscript{6)}

Alpha-mangostin (AMG) is one of the most studied xanthones from \textit{Garcinia mangostana}. It can be isolated from the pericarp of the mangosteen fruit.\textsuperscript{7)} AMG has drawn much interest because of its numerous pharmacological properties, including antioxidant, antitumor, anti-inflammatory, antiallergic, antibacterial, antifungal, antiviral, and antimalarial effects.\textsuperscript{7)} However, its poor aqueous solubility is a major obstacle for formulation development.\textsuperscript{8)}

Therefore, reduction in AMG particle size was performed using HPH. In this study, we aimed to investigate the effects of stabilizers and HPH-processing parameters, i.e. cycle number and pressure, on the particle size of AMG. Percent size reduction efficiency of AMG suspensions at different HPH conditions was determined.

\textbf{Experimental}

\textbf{Materials}

AMG was purchased from Xi'an Huarui Bio-Engineering Co., Ltd., China and used as
received. Polyvinylpyrrolidone K30 (PVP K30, Kollidon® 30), poloxamer 188 (P188, Kolliphor® P188), and polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (SLP, Soluplus®) were obtained from BASF, Germany. Sodium lauryl sulfate (SLS), hydroxypropyl methylcellulose (HPMC, Benecel™ K4M Pharm), and polyethylene glycol (PEG) 4000 were supplied by Ajax Finechem Pty Ltd., Australia; Ashland, Netherlands; and Mosselman, Belgium, respectively.

**Solubility Study**

The solubility study was performed by adding an excess amount of AMG in different aqueous stabilizer systems (0.5 % w/v). The stabilizers used in this study were PVP K30, P188, SLP, SLS, HPMC, and PEG4000. The mixture was shaken continuously using an incubator shaker (LSI-3016R, Daihan Labtech, India) at 30 ± 0.5 °C at 100 rpm for 24 h. The solution was filtered through a 0.45-μm syringe filter. The AMG content in the filtrate was analyzed using a UV-Vis spectrophotometer (UV-2600, Shimadzu, Japan) at 317 nm.

**Preparation of AMG Suspension by HPH**

From the solubility study, stabilizers showing solubility-enhancing effect for AMG were selected to prepare AMG suspensions. AMG was dispersed in deionized water containing 0.5 % (w/v) stabilizer to obtain a concentration of 0.3 % (w/v) using magnetic stirring. The mixture was sonicated for 20 min and then homogenized using a high-speed homogenizer (Ultra-Turrax T25, IKA, Germany) at 8,000 rpm for 10 min, followed by degassing in a sonicator. The particle size of AMG was reduced by passing through a piston-gap high-pressure homogenizer (APV-2000, SPX Flow Technology, UK). First, premilling steps were carried out at different pressures: 100, 300, 500, and 750 bar for 10 cycles at each stage. Then, milling steps were performed at 1,000-1,500 bar for 10-40 cycles. The sample temperature was controlled by using an external ice bath during processing. The samples were collected from time to time for particle size analysis and zeta potential measurement.
Particle Size and Size Distribution Measurement

The particle size of AMG suspension was determined using a laser diffraction particle size analyzer (Mastersizer 2000, Malvern, UK). The sample was added into the dispersion unit, and water was used as dispersant. Laser intensity was set at 10-15% during measurement. All samples were measured in triplicate. The particle size by the volume distribution was expressed as $D_{0.5}$. The distribution of particle size was expressed as a span calculated according to the following equation:

$$\text{Span} = \frac{(D_{0.9} - D_{0.1})}{D_{0.5}}$$

where $D_{0.9}$, $D_{0.5}$, and $D_{0.1}$ are particle diameters representing 90, 50, and 10 % of the population, respectively.

Zeta Potential Measurement

Zeta potential of the sample was measured using a photon correlation spectrophotometer (Zetasizer Nano ZS, Malvern, UK) at 25 °C. All samples were measured in triplicate.

Field Emission Scanning Electron Microscopy (FESEM)

The morphology of AMG powder was determined by FESEM (JSM-7610F FEG-SEM, JEOL, USA). The sample was mounted on an aluminum stub using a double-sided adhesive carbon tape. The stub was sputter-coated with gold and then photographed at different magnifications.

Statistical Analysis

Minitab® software version 16 (Minitab Inc., USA) was used for statistical analysis of the data. Analysis of variance (ANOVA) was used at a significance level of 0.05.
Results and Discussion

Solubility Study

The solubility of AMG in various 0.5 % (w/v) stabilizer solutions was measured to investigate the solubility-enhancing effects of each stabilizer. The solubility of AMG in water could not be determined owing to its very poor wetting property. The AMG powder was difficult to get wet with water and remained floating on the water surface. The solubility of AMG in various stabilizers was as follows: 57.81 ± 0.21 μg/mL for SLP, 45.26 ± 0.18 μg/mL for SLS, 6.45 ± 0.1 μg/mL for P188, and 2.3 ± 0.05 μg/mL for HPMC. Concentration of AMG in PEG4000 and PVP K30 solutions could not be determined by UV-VIS spectrophotometry, suggesting that PEG4000 and PVP K30 could not improve the aqueous solubility of AMG. Among the stabilizers used, SLP, SLS, P188, and HPMC could enhance the aqueous solubility of AMG. According to the solubility results, the aqueous solubility of AMG was higher in surfactants (SLS) than in polymeric stabilizers, except for SLP. With the use of surfactants, the surface tension between particles was reduced, thereby, particles could be wetted more easily.\(^9\) The solubilizing effects of SLP and P188 might be attributed to the formation of polymeric micelles since their concentration in the solubility study was above their critical micelle concentration (CMC) (0.082 and 0.4% (w/v) for SLP\(^{10}\) and P188\(^{11}\), respectively). Therefore, SLP with lower CMC could enhance the aqueous solubility of AMG more than P188.

Effect of Stabilizers and Homogenization Cycle Number on AMG Suspension by HPH

Figure 1A shows that the mean particle size of intact AMG was 9.8 ± 0.53 μm. The span was 3.49 ± 0.23, indicating wide size distribution. The morphological characterization of intact AMG by FESEM was shown in Fig. 1B. Intact AMG existed as a rod-shaped crystalline powder.

AMG suspensions with different types of stabilizers, including SLP, SLS, and P188,
were prepared by HPH at 1,000 bar and various homogenization cycles. Before HPH, AMG suspensions with different stabilizers had an initial particle size in the range of 6-9 µm with a span of 2.37-2.65 (Fig. 2A). The particle sizes of AMG suspensions with the stabilizers were significantly smaller than that of intact AMG \((p < 0.05)\). This could be attributed to the improved wetting and dispersion of AMG particles by the stabilizer. After HPH, the particle size of the AMG suspension was significantly reduced, compared to that before size reduction \((p < 0.05)\). Span values of all formulations were 1.26-1.99. Micronized AMG suspensions were successfully prepared. For each stabilizer, the particle size significantly decreased with the increase in homogenization cycle number \((p < 0.05)\). This could be explained using the Bernoulli’s theorem. The zone of good dispersion capability is considered to be in the center of the homogenization gap because it provides the highest streaming velocity. With the increase in the number of cycles, the probability that larger particles pass this zone increases; hence, the particle size becomes smaller.

Various particle sizes of AMG suspension were obtained when different stabilizers were used at different number of cycles (Fig. 2A). Among the stabilizer used, the smallest particles of AMG were obtained when SLS was used as a stabilizer \((p < 0.05)\). To determine the effect of stabilizers on the size of AMG particles, percent size reduction efficiency was calculated according to the following equation.

\[
\text{Percent size reduction efficiency} = \left( \frac{\text{mean particle size before HPH} - \text{mean particle size after HPH}}{\text{mean particle size before HPH}} \right) \times 100
\]

Percent size reduction efficiency of each stabilizer increased with the increase in homogenization cycle number (Fig. 2B). Although SLS showed lower percent size reduction efficiency than P188, the smallest particles for every 10-cycle step were obtained for the AMG-SLS suspension. This might be attributable to the smaller particle size of the suspension at the beginning. The higher the number of homogenization cycles, the smaller
the particle size. However, there is no proportional relationship between the decrease in size and the increase in homogenization cycle number. For size reduction by milling, the particles/crystals break more easily at the flaws or imperfections. By decreasing the particle size, the number of flaws gradually decreases. The remaining crystals become increasingly perfect. Hence, the force required to break the crystals increases with the decrease in size.\(^2\) In size reduction by HPH, force is primarily derived from cavitation, and secondarily from high-shear forces and interparticle collisions.\(^{12}\)

Stabilizers in suspensions play important role in the thorough wetting of the drug particles and stabilization of the particles by electrostatic or steric stabilization.\(^{13}\) During homogenization, the degree of dispersion of drug particles can influence the size reduction efficiency. If particles are staying apart during passing the narrow homogenization gap, a more efficient particle size reduction can be achieved.\(^9\) As shown in Fig. 2, SLS and P188 provided a greater extent of particle size reduction than that of SLP since they exhibited more effective wetting properties and consequently resulted in better dispersion of the particles. This is consistent with the general concept that surfactants could provide more effective wetting properties with better dispersion of particles and a greater extent of particle size reduction than that provided by polymeric stabilizers.\(^9\)

The zeta potential of AMG suspensions after HPH at 1,000 bar for 40 cycles was as follows: AMG-SLS, \(-66.6 \pm 0.6\) mV; AMG-P188, \(-38.6 \pm 0.7\) mV; and AMG-SLP, \(-26.7 \pm 0.6\) mV. The different values of zeta potential among the stabilizers might be owing to the nature of stabilizers and hence their stabilization mechanisms. Higher zeta potential value indicated the higher stability of suspensions owing to electrostatic stabilization. Zeta potential of AMG suspension with SLS (anionic surfactant) was high enough to provide electrostatic stabilization because a minimum value of \(\pm 30\) mV is required to obtain a stable formulation.\(^{14}\) However, suspensions containing nonionic stabilizers, SLP and P188, which
provided steric stabilization, had lower zeta potential value than that containing ionic surfactant. Generally, steric stabilization can maintain stable suspensions even with low zeta potential values if the stabilizers have good adsorption on the drug particles, and the dispersion medium is a good solvent that can maintain adsorbed stabilizers.15)

HPH is one of the promising methods used to prepare nanosuspensions.12) Regarding AMG, the micronized AMG-SLS suspension was successfully prepared by HPH at 1000 bar and 40 homogenization cycles. Percent size reduction efficiency increased with the increase in homogenization cycle number. Therefore, the effect of increasing the homogenization cycles to 80 cycles was further investigated. As shown in Fig. 3, the particle sizes of all formulations decreased, compared to those of the formulations at 40 cycles (p < 0.05). Span values were 1.8-2.44. Percent size reduction efficiency of AMG-SLS, AMG-P188, and AMG-SLP suspensions at 80 cycles was 86.9, 85.1, and 78%, respectively, implying that further increase in cycle number could decrease particle size. The AMG-SLP suspension showed bimodal size distribution, indicating clearly different particle sizes in the suspension. Only the mean particle size of the AMG-SLS suspension was in the nanosized range; however, larger particles still existed. These results suggest that SLS was more effective than P188 and SLP in particle size reduction.

**Effect of Homogenization Pressure on AMG Suspensions with Binary Stabilizer**

A micronized (not nanosized) suspension was obtained using a single stabilizer and homogenization pressure of 1000 bar for 80 cycles. Therefore, the effects of binary stabilizer system, such as 0.5 % (w/v) SLS and 0.5 % (w/v) P188, and higher pressure (1500 bar) were further investigated. This was expected to result in more efficient particle size reduction. Results are shown in Table 1. The mean initial particle size of AMG suspension with binary stabilizer system was 7.8 ± 0.29 µm with a span of 2.39 ± 0.02. The particle size of AMG suspension with binary stabilizer system was significantly different from that of suspensions
with a single stabilizer ($p < 0.05$). Binary stabilizer system showed better degree of dispersion of drug particles than that of the polymeric stabilizer alone. After size reduction, the suspension with binary stabilizer system showed a similar trend to that of the suspensions with a single stabilizer regarding the decrease in particle size with the increase in homogenization cycles. Percent size reduction efficiency at 1500 bar was higher than that at 1000 bar for every 10-cycle step. The particles prepared by higher pressure were significantly smaller than those obtained at lower pressure ($p < 0.05$). It could be concluded that high homogenization pressure was required to achieve a smaller particle size. However, it is noteworthy that there was no linear relationship between the decrease in size and increase in pressure. When the homogenization pressure was increased up to 4000 bar, a relatively small further decrease in particle size was observed.

In conclusion, the increase in the homogenization pressure and number of cycles, as well as using a binary stabilizer system were not able to reduce the particle size of AMG to the nanometer range. This could be because of the hardness of the drug. AMG is a crystalline substance with high intensity. Manually ground AMG for 60 min showed no transformation from crystalline to amorphous form, as confirmed by the powder X-ray diffraction pattern (in the supplementary data).

**Conclusions**

In this study, the effects of stabilizers, homogenization cycle number, and pressure on size reduction of AMG using HPH were investigated. SLS, P188, and SLP could be used to enhance the aqueous solubility of AMG. Micronization of AMG particles was achieved by HPH. It was found that surfactants were more effective than polymeric stabilizers in particle size reduction because of the better wetting and dispersion of AMG particles. Increasing homogenization cycle number and pressure significantly decreased the size of AMG particles.
Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Materials

The online version of this article contains supplementary materials.
References

**Figure 1** Particle size and size distribution (A) and SEM image (B) of intact AMG
Figure 2 (A) Particle size and size distribution of AMG suspensions containing stabilizers before and after size reduction by HPH at 1,000 bar with different number of cycles (B) Percent size reduction efficiency of AMG suspensions prepared by HPH at 1,000 bar with 10-40 cycles
Figure 3 Particle size distribution of AMG suspensions prepared by HPH at 1,000 bar with 80 cycles: (A) AMG-SLS, (B) AMG-SLP, and (C) AMG-P188
**Table 1** Particle size and size distribution of AMG suspensions with a binary stabilizer system (0.5 % w/v SLS and 0.5 % w/v P188) prepared by HPH under different conditions

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<th>Pressure (bar)</th>
<th>Cycles</th>
<th>( D_{0.1} ) (μm)</th>
<th>( D_{0.5} ) (μm)</th>
<th>( D_{0.9} ) (μm)</th>
<th>Span</th>
<th>% Size reduction efficiency</th>
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<td>1000</td>
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