Supporting Information

Fabrication, Characterization and Formation Mechanism of Zein/Gum Arabic Nanocomposites in Aqueous Ethanol Solution with High Ethanol Content

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Purification of α-Zein. Commercial zein was treated to obtain the purified α-zein according to the reported method from Paraman and Lamsal ¹ with some modifications. Briefly, 25 g of zein powders were suspended in 100 mL aqueous ethanol solution (90 vol % ethanol) and vigorously stirred for 1 h at room temperature. The insoluble portion was removed by centrifugation at 1000 g for 20 min. The resultant supernatant was kept at 4 °C overnight and centrifuged again at 1000 g for 20 min to remove the insoluble portion. Cold distilled water (15 °C) was added to precipitate the protein with a volume ratio of zein aqueous ethanol solution to distilled water of 1:4. The suspension was centrifuged at 3000 g for 20 min, and the precipitates were frozen at -80 °C and dried for 72 h with a freeze-drying apparatus (Scientz-18N, Ningbo, China). The obtained purified α-zein powders were stored at a desiccator for further use after passed through a 60 mesh sieve.

SDS-PAGE Analysis. Based on the previously published method,² the molecular weight profile of the purified α-zein was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions using commercial 4-20% Mini-PROTEAN TGX™ Precast Protein Gels (BioRad,
Hercules, CA). The sample buffer (BioRad, Hercules, CA) contains 62.5 mM Tris-HCl at pH 6.8, 2% SDS, 25% glycerol, and 0.01% bromophenol blue. The running buffer (BioRad, Hercules, CA) contains 25 mM Tris at pH 8.3, 0.1% SDS, and 192 mM glycine. The extracted α-zein was dissolved in 70% (v/v) aqueous ethanol and diluted to 2 μg protein/μL with the sample buffer, and 10 μL of the soluble protein was loaded onto the gel. Electrophoresis was performed at a constant voltage of 200 V for 40 min. The gel was stained by 0.1% Coomassie brilliant blue solution. Bio-Rad protein markers with a molecular weight ranging from 10 to 200 kDa were used.

Figure S1. Protein profile of α-zein fractions isolated from commercial zein (A), and photographs of zein before and after purification (B).

Figure S1A shows the electrophoretic profile of purified zein at a concentration of 2.0 mg/mL. Two intense bands with apparent migration rates (Mr) of 19 and 22 kDa were observed on SDS-PAGE, indicating that the treated zein consisted largely of α-zein monomer. The results were consistent with the previous report from Anderson and Lamsal, where they extracted α-zein with the molecular weight (M_w) of 19,000
and 22,000 Da from corn gluten meal. There was a faint band at about 45 kDa, which was proved to be the dimer of α-zein through disulfide-bridges.\(^5\) In addition, the initial yellowness of commercial zein faded after purification as shown in photographs (Figure S1B), suggesting that pigments in zein, such as carotenoids, lutein and zeaxanthin, were removed.\(^6\)

**References**


Table S1. The mean size of Zein-GA nanocomposites with mass ratios of zein to GA of 10:1, 5:1, 2:1, 1:1 and 1:2

<table>
<thead>
<tr>
<th></th>
<th>Zein</th>
<th>Zein-GA&lt;sub&gt;10:1&lt;/sub&gt;</th>
<th>Zein-GA&lt;sub&gt;5:1&lt;/sub&gt;</th>
<th>Zein-GA&lt;sub&gt;2:1&lt;/sub&gt;</th>
<th>Zein-GA&lt;sub&gt;1:1&lt;/sub&gt;</th>
<th>Zein-GA&lt;sub&gt;1:2&lt;/sub&gt;</th>
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<tr>
<td>Mean size (nm)</td>
<td>561.30±56.44&lt;sup&gt;a&lt;/sup&gt;</td>
<td>180.38±15.40&lt;sup&gt;b&lt;/sup&gt;</td>
<td>172.02±5.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>177.60±16.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>234.22±7.30&lt;sup&gt;c&lt;/sup&gt;</td>
<td>351.98±6.16&lt;sup&gt;d&lt;/sup&gt;</td>
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Figure S2. Fluorescence spectrum of GA at different concentrations

Figure S2 shows the narrowed scale of Y-axis for GA alone, which may make it clearly to read.