Supporting Information


Plant-Based Hollow Microcapsules for Oral Delivery Applications: Toward Optimized Loading and Controlled Release

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Figure S1. Scanning electron microscope images of sunflower sporopollenin exine capsules (SECs) following different durations of H₃PO₄ acidolysis: (A) without acidolysis; (B) 1 h; (C) 5 h; (D) 10 h; (E) 20 h.
Figure S2. Confocal microscopy analysis of sunflower sporopollenin exine capsules (SECs) following varying durations of H$_3$PO$_4$ acidolysis. (Scale bars 10 µm).
Figure S3. Residual protein content in sunflower sporopollenin exine capsules (SECs) following different periods of H₃PO₄ acidolysis, as measured with CHN elemental analysis. Data is presented as the average of triplicate measurements with standard deviation (n = 3).
Figure S4. Large molecule content analysis by MALDI-TOF for sunflower pollen and sporopollenin exine capsules (SECs) based on sample grinding and ethanol extracts: (A) Mass-to-charge ratio spectrum for pollen, SECs, and MALDI matrix (CHCA) from 1000 m/z to 20000 m/z; (B) Major mass-to-charge ratio peaks for SECs; (C) Major mass-to-charge ratio peaks for pollen; and (D) Relative peaks heights of major pollen peaks, CHCA peaks, and major SEC peaks. Data is presented as representative of triplicate measurements.
Figure S5. Characterization of sunflower sporopollenin exine capsules (SECs) before and after extraction using phosphoric acid at varying time points: (A) Phosphoric acid treated SECs; (B) Only intact SECs.
Figure S6. Optical micrographs of sunflower pollen grains and SECs produced by varying periods of H$_3$PO$_4$ acidolysis.
Figure S7. Scanning electron microscopic images of BSA-loaded sunflower sporopollenin exine capsules (SECs) with varying BSA-loading proportions: (A) 23 wt.%; (B) 37.5 wt.%; and (C) 54.5 wt.% (Scale bars 10 μm).

Figure S8. Micromeritic properties of BSA-loaded sunflower SECs: (A) diameter (µm); (B) circularity; (C) aspect ratio; for 1000 sunflower SECs prior and following BSA-loading. The data obtained by the spline curve fitting of histogram data from triplicate batches (n = 3).
Figure S9. Confocal laser scanning microscopy (CLSM) Z-stack analysis of sunflower SECs before and after FITC-BSA loading at three different BSA-loading proportions.
Figure S10. Confocal laser scanning microscopy (CLSM) of sunflower SECs before and after FITC-BSA loading at three different BSA-loading proportions: (A) Unloaded SECs, and FITC-BSA loading at three different loadings; (B) Quantification of fluorescence intensity from FITC-BSA loaded SECs at three different BSA-loading proportions.
Figure S11. Scanning electron microscopic images of sunflower SECs following their in vitro release SGF and SIF. (Scale bars 10 µm).

Table S1. Sunflower sporopollenin exine capsules (SECs): CHN composition\textsuperscript{a)}

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Carbon [%]</th>
<th>Hydrogen [%]</th>
<th>Nitrogen [%]</th>
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<tr>
<td>Defatted</td>
<td>48.8 ± 0.2</td>
<td>7.6 ± 0.2</td>
<td>5.2 ± 0.0</td>
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<tr>
<td>H\textsubscript{3}PO\textsubscript{4} acidolysis 1 h</td>
<td>60.4 ± 0.0</td>
<td>8.0 ± 0.0</td>
<td>1.1 ± 0.0</td>
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<tr>
<td>H\textsubscript{3}PO\textsubscript{4} acidolysis 5 h</td>
<td>61.5 ± 0.7</td>
<td>7.5 ± 0.6</td>
<td>0.7 ± 0.0</td>
</tr>
<tr>
<td>H\textsubscript{3}PO\textsubscript{4} acidolysis 10 h</td>
<td>62.1 ± 0.6</td>
<td>7.5 ± 0.5</td>
<td>0.6 ± 0.0</td>
</tr>
<tr>
<td>H\textsubscript{3}PO\textsubscript{4} acidolysis 20 h</td>
<td>61.8 ± 1.4</td>
<td>6.8 ± 1.6</td>
<td>0.6 ± 0.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a)}CHN analysis performed in triplicate and reported as average values with standard deviation
Table S2. Equivalent spherical diameter of sunflower SECs\(^a\)

<table>
<thead>
<tr>
<th>Sunflower</th>
<th>Equivalent spherical diameter (ESD) [µm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defatted pollen grains</td>
<td>37.0 ± 1.5</td>
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<tr>
<td>(\text{H}_3\text{PO}_4) acidolysis 1 h</td>
<td>27.8 ± 1.3</td>
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<tr>
<td>(\text{H}_3\text{PO}_4) acidolysis 5 h</td>
<td>30.2 ± 1.1</td>
</tr>
<tr>
<td>(\text{H}_3\text{PO}_4) acidolysis 10 h</td>
<td>30.3 ± 0.8</td>
</tr>
<tr>
<td>(\text{H}_3\text{PO}_4) acidolysis 20 h</td>
<td>30.3 ± 0.8</td>
</tr>
</tbody>
</table>

\(^a\)DIPA analysis performed in triplicate and reported as average values with standard deviation

Table S3. Estimation of SEC theoretical maximum BSA-loading efficiency\(^a\)

<table>
<thead>
<tr>
<th>BSA Loading Achieved</th>
<th>Multiplication factor required to achieve 100% full SECs</th>
<th>Theoretical Maximum BSA Loading</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BSA weight proportion to SECs [BSA : SECs]</td>
<td>BSA loading achieved [vol. %]</td>
</tr>
<tr>
<td>9.4</td>
<td>0.104</td>
<td>12.7</td>
</tr>
<tr>
<td>15.8</td>
<td>0.188</td>
<td>22.9</td>
</tr>
<tr>
<td>22.3</td>
<td>0.287</td>
<td>29.5</td>
</tr>
<tr>
<td>Average</td>
<td></td>
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</tbody>
</table>

\(^a\)Loading analysis based on data from triplicate batches
Table S4. Details of control study tablets $^{a)}$

<table>
<thead>
<tr>
<th>Tableted formulation</th>
<th>Weight of tablet [mg]</th>
<th>Diameter of tablet [mm]</th>
<th>Thickness of tablet [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>No SECs – before coat</td>
<td>149.8 ± 2.2</td>
<td>12.92 ± 0.01</td>
<td>0.95 ± 0.01</td>
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<tr>
<td>No SECs – after coat</td>
<td>175.5 ± 0.7</td>
<td>12.99 ± 0.01</td>
<td>1.32 ± 0.04</td>
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<tr>
<td>BSA SECs – no coat</td>
<td>150.1 ± 1.3</td>
<td>12.92 ± 0.01</td>
<td>1.08 ± 0.01</td>
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</table>

$^{a)}$Tablets prepared using 13 mm stainless steel press die punch and results represent the mean of three batches (n=3) with standard deviations.