Preparation of Biopolymer Aerogels Using Green Solvents

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Abstract

Although the first reports on aerogels made by Kistler in the 1930s dealt with aerogels from both inorganic oxides (silica and others) and biopolymers (gelatin, agar, cellulose), only recently have biomasses been recognized as an abundant source of chemically diverse macromolecules for functional aerogel materials. Biopolymer aerogels (pectin, alginate, chitosan, cellulose, etc.) exhibit both specific inheritable functions of starting biopolymers and distinctive features of aerogels (80-99% porosity and specific surface up to 800 m²/g). This synergy of properties makes biopolymer aerogels promising candidates for a wide gamut of applications such as thermal insulation, tissue engineering and regenerative medicine, drug delivery systems, functional foods, catalysts, adsorbents and sensors. This work demonstrates the use of pressurized carbon dioxide (5 MPa) for the ionic cross linking of amidated pectin into hydrogels. Initially a biopolymer/salt dispersion is prepared in water. Under pressurized CO₂ conditions, the pH of the biopolymer solution is lowered to 3 which releases the crosslinking cations from the salt to bind with the biopolymer yielding hydrogels. Solvent exchange to ethanol and further supercritical CO₂ drying (10 - 12 MPa) yield aerogels. Obtained aerogels are ultra-porous with low density (as low as 0.02 g/cm³), high specific surface area (350 - 500 m²/g) and pore volume (3 - 7 cm³/g for pore sizes less than 150 nm).

Introduction

Aerogels are a class of porous materials that can be prepared using a variety of precursors ranging from inorganic (such as silica, titania, zirconia and others), synthetic (such as resorcinol formaldehyde, polyurethane and others) or biopolymers (polysaccharides, proteins and others). What sets them apart from the conventional porous materials is their ability to simultaneously possess all the three characteristics; namely high surface area, ultra-low density and mesoporous pore size distribution (i.e., pore sizes from 2-50 nm). With aforementioned characteristics, aerogels are extensively applied in the fields of insulation, biomedicine, catalysis, adsorption and absorption applications, pharmaceuticals and neutraceuticals. Taking into account the above possibilities, the production of biopolymer gel systems and their subsequent transformation to aerogels opens up a multitude of opportunities towards high value added bio based materials. Such an endeavor is taken up in this study using amidated pectin as an example.

Aerogels are typically produced by the sol-gel technique. Gels are systems consisting of liquid entrapped in a matrix and can be prepared by covalent, ionic, pH induced, thermal or cryo cross linking. For this specific system, we utilize ionic crosslinking; i.e., a bivalent cation (e.g., calcium) to crosslink biopolymeric chains together. To perform controllable ionic crosslinking of biopolymers such as amidated pectin or alginate, one can utilize the diffusion method or the internal setting method. In the diffusion method, gelation occurs at first in the outer layer followed by diffusion propagation, as the cations diffuse from outer solution into an amidated pectin or alginate droplet or layer. In the internal setting method, the insoluble form of the crosslinker is homogenously dispersed in the biopolymer solution and cations are released by initiating a pH change. However, both techniques face an issue regarding the homogeneity of the final gel when produced in slab or monolithic form. This can be circumvented by using pressurized CO₂ (5 MPa) for the production of amidated pectin hydrogels building further on previous works on calcium carbonate solubilize, releasing the calcium ions. The calcium ions crosslink with the amidated pectin biopolymer to yield hydrogels. Stable homogeneous gels down to very low biopolymer concentrations (0.05 wt%) could be produced using this technique.

As gelation takes place in an aqueous medium, solvent exchange to an organic solvent is required due to a miscibility gap in the CO₂/water system. Typically low molecular weight alcohols (methanol/ethanol/isopropanol) and ketones (acetone) can be used for the solvent exchange process. However, direct soaking in a bath with pure ethanol or other organic solvents leads to significant irreversible shrinkage. To avoid this drawback, stepwise solvent exchange is performed. When the solvent concentration inside the gel reaches >98%, the organic solvent is dried with supercritical CO₂ (12 MPa) leaving behind an aerogel.
Protocol

1. Preparation of Amidated Pectin Stock Solution

1. Mix 20 g amidated pectin with 980 g water (2.0 wt%). The degree of amidation is 25 wt%.
2. Homogenize the solution with a high speed stirrer (10,000 rpm) for 2 min to obtain a homogenous viscous solution.
3. Measure pH using pH strips or pH meter. If the pH is lower than 6.5, titrate with 0.5 M NaOH to neutralize the solution (to pH 7.0).
4. Add calcium carbonate in a ratio of 0.1825 g per gram of dry amidated pectin (q=1). “q” denotes the degree of crosslinking.
5. For 1 kg 2.0 wt% amidated pectin solution, add 3.65 g calcium carbonate (q=1) per 20.0 g dry pectin.
6. For greater crosslinking, add 0.3650 g calcium carbonate per gram of dry amidated pectin (q=2).

2. Production of Hydrogels

1. Homogenize the amidated pectin/calcium carbonate mixture using the high speed homogenizer (10,000 rpm) to obtain a white homogenous dispersion.
2. Transfer the suspension into open polypropylene molds or glass Petri dishes.
3. Place the molds in the high pressure autoclave. Seal the autoclave.
4. Pressurize the autoclave with gaseous CO\textsubscript{2} up to 5 MPa at RT. Refer to Gurikov et al.\textsuperscript{7} for further information. Maintain the pressure for 24 hr.
5. Slowly depressurize the autoclave at 0.2 MPa/min.
6. Open the autoclave and remove the molds. Remove the hydrogels from the molds by turning them over. If necessary, use a spatula.

3. Solvent Exchange Procedure

1. Prepare 10 g of 10:90 (w/w) ethanol/water mixture per gram of hydrogel.
2. Immerse the hydrogels in the 10:90 (w/w) ethanol/water mixture for 12 hr.
3. Continue this process with increasing ethanol concentrations, i.e., from 10:90 (w/w) ethanol/water mixture to 30:70 (w/w) ethanol/water mixture. After 12 hr, transfer to 50:50 (w/w) ethanol/water mixture, then to 70:30 (12 hr), then 90:10 (12 hr) and then to 100% ethanol solution (12 hr).
4. Soak the gel further in pure ethanol so that the final concentration inside the gel is more than 98% (w/w). Measure the concentration using the density meter. The alcogel is now ready for supercritical CO\textsubscript{2} drying.

4. Production of Aerogels by Supercritical CO\textsubscript{2} Drying

1. Place the samples in the same high pressure autoclave used for hydrogel preparation (see step 2.3).
2. Fill the autoclave with additional ethanol (2-10% of the autoclave volume) to prevent premature solvent evaporation from the gels. Complete immersion of gel in the solvent is not required.
3. Seal the autoclave. Turn on the autoclave heating. Set the autoclave working temperature to 323 K. Pressurize the autoclave with carbon dioxide to 12 MPa using a compressor or pump.
4. Periodically replace the CO\textsubscript{2} inside the autoclave\textsuperscript{10,11} with fresh CO\textsubscript{2} keeping the pressure constant. 6-7 residence volumes are required over a period of 6 hr. Refer to Gurikov et al.\textsuperscript{7} for further information.
5. Slowly depressurize the autoclave at 0.2 MPa/min.
6. Open the autoclave and collect the aerogel. Store the aerogel in an excicator or a sealed container.

Representative Results

The typical hydrogels obtained after gelation step with higher crosslinking degree (q=2) (as instructed in Protocol section 2) are shown in Figure 1. The samples on the left (sample A and B) are the 2 wt% and 1 wt% pectin gels obtained by CO\textsubscript{2} induced gelation. By decreasing the biopolymer concentration (0.5 wt% or lower), the gels become transparent (sample C). Further reduction in the biopolymer concentration (0.25 wt%) also yields stable hydrogels (sample D) but these gels are very fragile and can break when handling. The bubbles observed inside the hydrogels are created during depressurization when the dissolved CO\textsubscript{2} leaves the gel water system due to decrease in CO\textsubscript{2} solubility.

The amidated pectin aerogel characteristics are presented in the Table 1. The obtained aerogels are ultra-porous with low density (as low as 0.013 g/cm\textsuperscript{3}) measured as the ratio between the mass of the aerogel and its volume. The surface area is measured by the nitrogen adsorption. For pectin aerogels, it yielded a specific surface area between 350 - 500 m\textsuperscript{2}/g. The pore volume for pore sizes in the 4-150 nm range is measured by the Kelvin model of pore filling using nitrogen (BJH method). The pore volume for the amidated pectin aerogels was between 3 - 7 cm\textsuperscript{3}/g for pore sizes between 4 and 150 nm.
Table 1. Characteristics of the amidated pectin aerogels.

<table>
<thead>
<tr>
<th>Pectin concentration [wt%]</th>
<th>Cross-linking degree q</th>
<th>Bulk density [g/cm³]</th>
<th>Specific surface area [m²/g]</th>
<th>Specific pore volume [cm³/g]</th>
<th>Average pore size (diameter) [nm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1</td>
<td>0.081</td>
<td>502</td>
<td>4.1</td>
<td>14</td>
</tr>
<tr>
<td>1.00</td>
<td>1</td>
<td>0.044</td>
<td>491</td>
<td>7.1</td>
<td>27</td>
</tr>
<tr>
<td>0.50</td>
<td>1</td>
<td>0.035</td>
<td>357</td>
<td>3.8</td>
<td>27</td>
</tr>
<tr>
<td>0.25</td>
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<td>0.013</td>
<td>335</td>
<td>4.9</td>
<td>41</td>
</tr>
<tr>
<td>2.00</td>
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<td>0.069</td>
<td>447</td>
<td>3.1</td>
<td>13</td>
</tr>
<tr>
<td>1.00</td>
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<td>0.048</td>
<td>441</td>
<td>3.6</td>
<td>26</td>
</tr>
<tr>
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<td>25</td>
</tr>
<tr>
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<td>2</td>
<td>0.017</td>
<td>347</td>
<td>5.0</td>
<td>24</td>
</tr>
</tbody>
</table>

Discussion

By using the CO₂-induced gelation technique, one can eliminate the need for chemical substitutes (for example acetic acid or glucono delta-lactone (GDL)) required for inducing the crosslinking of the biopolymer. The surface areas of the amidated pectin aerogels are in the higher ranges of literature values⁵, however the pore volumes are much higher than those presented in literature⁶. Higher pore volumes were also
observed for alginate aerogels prepared by CO₂ induced gelation. However, it remains to be verified whether the reason for this high pore

volumes (4-150 nm pore size range) is due to the gelation technique or an inherent property of the biopolymers previously not addressed

in literature. Pectin aerogels have been reported in literature to possess superinsulating properties and alginate aerogels prepared by this

technique also possess thermal conductivities in the superinsulating range. Therefore, the amidated pectin aerogels produced by this

technique may also be envisaged to possess superinsulating properties.

The rate of depressurization in Protocol Section 2 is an important step in the hydrogel preparation. Fast depressurization can lead to increased

macroporosity of the gels. This phenomena can be applied for tissue engineering applications where macroporosity of the material with

interconnectivity is an important feature for the growth and proliferation of cells. In addition, the crosslinking degree in Protocol Section 1

plays an important role in the synergy and swelling property of the amidated pectin hydrogels. This is similar to alginate hydrogels whose

swelling behavior is influenced by the crosslinker concentration as well. Thereby aerogels made by amidated pectin can also be tuned to

possess superbabsorbent property similar to those reported for alginate aerogels.

By using the CO₂ induced gelation considering amidated pectin (or alginate) as the primary system, further diversity can be incorporated into

the aerogels by introducing different cross linking agents and biopolymer combinations. Several metal carbonates (e.g., zinc, nickel, cobalt,
copper, strontium, barium) could be used for cross-linking, where cations can be released by pH lowering in aqueous media with pressurized

CO₂ (3-5 MPa). However, insoluble salts of some of these cations may not form stable dispersions for lower biopolymer concentrations and can settle down at the bottom leading to inhomogeneous gels. This is a general issue with the internal setting gelation method including CO₂ induced gelation and thereby the technique’s usability for an application should be evaluated on a case to case basis.

Various blends prepared using water soluble biopolymers such as starch, carrageenan, methyl and carboxy methyl cellulose, gellan gum, lignin,
gelatin and others; water soluble synthetic polymers such as polyethylene glycol (PEG), polyvinyl alcohol (PVA), Pluronic P-123 and others; and

water soluble inorganic precursors such as sodium silicate can be also mixed with amidated pectin to produce hybrid aerogels similar to alginate

with tunable properties.

As supercritical CO₂ drying (scCO₂ drying) is a quintessential step in aerogel production, any combination of pre-processing steps such as solvent exchange and drying using CO₂, or gelation, solvent exchange and drying using CO₂ could provide a clear processing advantage. The advantage is envisioned as integrated one pot process: where biopolymer dispersions can be converted into biopolymer aerogels using CO₂ as the main processing medium in a single autoclave. For certain pharmaceutical applications, one can also envision performing a four step: gelation, solvent exchange, supercritical drying and active component loading process in a single autoclave using CO₂ as the processing medium. Post treatment such as protective coating of drug loaded aerogels in certain cases is necessary for targeted drug release.

To conclude, the present work demonstrates the use of pressurized CO₂ for gelation of amidated pectin based systems. In addition, the use of pressurized CO₂ as a common medium for precursor to product conversion for target applications in a single autoclave is envisaged.