

Supercritical Gel Drying for the Production of Starch Aerogels for Delivery Systems

Iolanda De Marco*, Lucia Baldino, Stefano Cardea, Ernesto Reverchon

University of Salerno, Department of Industrial Engineering, Via Giovanni Paolo II, 132, 84084, Fisciano (SA), Italy
 idemarco@unisa.it

Supercritical fluids based processes were frequently proposed to realize new kind of supports to deliver poor water-soluble drugs. In some cases, nanostructured aerogels were used as substrates. The matrices constituting the aerogel have to present a high porosity and a high surface area. Different natural and synthetic polymers were used in form of aerogels. Natural polymer-based materials are preferable for their renewability and for environmental reasons; among them, starch is the second most abundant after cellulose and it is attractive for its low cost. In this work, different sources starches (maize, potato and wheat) aerogels were produced using a supercritical carbon dioxide based process. First, a gel is formed by an aqueous solution (hydrogel), then water is replaced by ethanol to form an alcogel; finally, a supercritical gel-drying step is proposed to form the aerogel. The different sources starches were processed and characterized from a macroscopic and a microscopic point of view. The analyses confirmed that the supercritical gel drying process is suitable to form polymeric nanostructured matrices that can be used for delivery systems.

1. Introduction

Several supercritical fluids based processes have traditionally been used to obtain micronized powders for life sciences applications (Hakuta et al., 2003). Depending on the role played by supercritical carbon dioxide (scCO₂), various techniques have been developed, enhanced and modelled (Marra et al., 2012); for example, in the rapid expansion of supercritical solutions (RESS), scCO₂ is the solvent for the material (Hezave and Esmailzadeh, 2010), in the gas antisolvent precipitation (GAS) (De Marco et al., 2013) and in the supercritical antisolvent precipitation (SAS) (Reverchon and De Marco, 2011) is the antisolvent, in the supercritical assisted atomization (SAA) (Reverchon and Antonacci, 2007) and in the particles from gas-saturated solutions (PGSS) (Shariati and Peters, 2003) is the co-solute and in the expanded liquid antisolvent precipitation (ELAS) is the co-antisolvent (Prosapio et al., 2014). These techniques, once properly chosen the operating conditions, assure a good control of particle size, particle size distributions and not-agglomeration of the particles, even at nanodimensions (Torino et al., 2010). Unfortunately, some characteristics, like porosity and internal surface area, are limited in nanoparticles and microparticles. These lacks stimulated the research advancement in the field of different materials production, like, for example, aerogels, that present high porosities (85-99 %) and large surface areas (up to 3,000 m²/g). These materials are commonly obtained from wet gels using a proper drying technique, like freeze-drying (Jin et al., 2008) or supercritical drying (Cardea et al., 2013). In the case of freeze-drying, it is difficult the tuning of pore sizes, because the kinetics of the thermal quenching process has a great influence on the hydrogel architectures formed using this method. Moreover, the structural stability of the aerogels obtained using this technique is low and the mechanical properties often required for the usage of the aerogels are generally weak. Finally, the matrix may collapse at the material-air interface due to the interfacial tension resulting from the solvent evaporation, generating a surface skin (Ho et al., 2004) and the requirements, in terms of energy and time consuming, to completely remove the solvent are high (Quirk et al., 2005). The scCO₂ gel drying process recently developed (Reverchon et al., 2008) realized the drying in a cheaper and less energy consuming way, maintaining the macro, micro and nanostructure of the gel. The usage of the aerogels in pharmaceutical, nutraceutical and

food industries is related to their biocompatibility and biodegradability. Silica aerogels have been frequently used with a wide range of applications in several fields, as for example to produce composites by supercritical adsorption of metals (Caputo et al., 2010) or of pharmaceutical products (Caputo et al., 2012), but they are biocompatible but not biodegradable (Smirnova et al., 2003). To overcome this limitation, natural polymer-based materials became good candidates for the formation of aerogels (Mehling et al., 2009). Among them, polysaccharides, due to their low toxicity, were indicated as key ingredients for the production of bio-based materials in food, nutraceutical and pharmaceutical fields (García-González et al., 2011). Starch is an abundant and low-cost polysaccharide; therefore, it can be used in drug delivery systems (Malafaya et al., 2006). Starch aerogel production was previously demonstrated and the effect of some critical parameters was investigated (García-González and Smirnova, 2013).

In this work, we further developed the processing of starch aerogels, studying the effect of starch source (maize, potato and wheat starch), time of solvent exchanging and starch concentration (5, 10 and 15 wt %) on the morphology of aerogels.

2. Materials and Methods

2.1 Materials

Potato starch S2004 (PS), wheat starch S5127 (WS), and ethanol (EtOH, purity 99.5 %) were purchased from Sigma-Aldrich (Italy); Maize starch 85652 (MS) was purchased from Fluka (Italy); carbon dioxide (purity 99%) was bought from SON (Società Ossigeno Napoli - Italy). Water was distilled using a laboratory water distiller supplied by ISECO S.P.A. (St. Marcel, AO, Italy). All materials were used as received.

2.2 Apparatus

Samples were prepared in a homemade apparatus that mainly consists of a 316 stainless steel cylindrical high-pressure vessel (i.V. = 80 mL), equipped with a high-pressure pump (Milton Roy, mod. Milroyal B, France) used to deliver the carbon dioxide. Pressure in the vessel was measured by a manometer (OMET, mod. 0.25, Italy) and regulated by a micrometering valve (Hoke, mod. 1335G4Y, SC, USA). Temperature was regulated by temperature controllers (Watlow, mod. 305, Italy). At the exit of the vessel, a rotameter (ASA, mod. D6, Italy) was used to measure the CO₂ flow rate.

2.3 Processing

The processing steps needed to produce an aerogel are summarized in Figure 1.

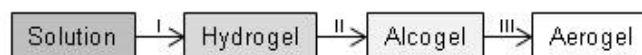


Figure 1: Scheme of the supercritical drying process for the attainment of the aerogels: (I) gelation; (II) solvent exchange; (III) supercritical drying

Aerogel processing starts with the gelation of starch (indicated with I in Figure 1), that involves melting the starch in an aqueous medium to induce changes in the structure (gelatinization step) and rearrange the structure during a cooling step (retrogradation step). Accordingly, the process starts with the preparation of the solutions (with starch concentrations equal to 5, 10 or 15 % wt) in distilled water; using a magnetic stirrer, the solutions were shaken until they became homogeneous (about 24 h). Then, they were heated up to 110 °C and poured into cylindrical moulds with an internal diameter of 2 cm and a height of 1 cm. Then, the samples were placed in the fridge for retrogradation at 4 °C for three days. The second step is the attainment of an alcolgel, replacing the water filling the pores of the gel structure by ethanol at room temperature (step II in Figure 1). The water in the hydrogel can be gradually replaced by ethanol by batch equilibration with a one-step ethanol bath (García-González and Smirnova, 2013) or with a succession of ethanol baths (Glenn and Stern, 1999). In particular, using the latter procedure, the dehydration occurs in subsequent ethanol-water baths at increasing ethanol concentration (30 %, 70 %, 90 % and two times 100 % (v/v)). For both the exchanging procedures, each ethanol bath contained two volumes of liquid for each volume of gel. The equilibration time for each bath was 2 or 24 h. The alcolgels are then dried using supercritical carbon dioxide (scCO₂) to form the aerogels (step III in Figure 1) using the following procedure: the vessel where the samples were placed was closed and filled from the bottom with scCO₂. When the desired pressure and temperature were reached (200 bar and 45 °C), drying was performed, fixing the scCO₂ flow rate at 1 kg/h, corresponding

to a residence time inside the vessel of about 4 min; the drying lasted 5 h. A slow depressurization (20 min) was used to bring back the system at atmospheric pressure and recover the aerogels from the vessel.

2.4 Characterizations

Scanning electron microscopy was used to evidence the micro and nanostructure of the supercritical dried aerogels. Samples were cryofractured using liquid nitrogen, put on a carbon tab previously stuck to an aluminium stub (Agar Scientific, Stansted, United Kingdom) and sputter coated with gold (Agar Scientific, Auto Sputter Coater, mod. 108 A, Stansted, United Kingdom) at 30 mA for 180 s. Then they were observed by a Field Emission Scanning Electron Microscope (FESEM, mod. LEO 1525, Carl Zeiss SMT AG, Oberkochen, Germany).

Thermograms of the aerogels were obtained using a Differential Scanning Calorimeter (DSC, mod. TC11, Mettler-Toledo Inc., Columbus, USA) using MettlerSTARe system. Fusion temperature and enthalpy were previously calibrated with indium standard materials (melting point 156.6 °C, enthalpy of fusion 28.52 J/g). Starch aerogel samples (5 ± 0.5 mg), prepared in duplicates, were accurately weighed, crimped into an aluminium pan and heated from 5 to 180 °C at 10 °C/min under a nitrogen purge (50 mL/min).

The residual moisture content for the different starch aerogels was determined by mass reduction by heating the aerogels at 110 °C for 24 h.

Surface area of the aerogels was characterized by ultra-high-purity nitrogen adsorption and desorption isotherms at 77 K using the Brunauer, Emmet and Teller (BET) method (Quantachrome Instruments, mod. Nova 1200e, Kingsville, TX). Prior to the measurement, about 200 mg of the sample was heated at 110 °C for 2 h under vacuum. For the determination of the surface area, adsorption isotherms in the linear region of the BET plot (at a relative pressure p/p_0 in the range 0.05-0.3) using a multipoint BET were evaluated.

3. Results and Discussion

3.1 Potato starch

Potato starch alcogel were prepared following the procedures described before, varying the concentration of starch in water (5-15 %) and the time for the solvent exchanging (2 h and 24 h). Then, they were dried by scCO_2 to obtain the corresponding aerogels. For all the tested concentrations, if the solvent exchanging time was chosen equal to 2 h, the structure obtained showed closed pores (as it can be seen from the FESEM image in Figure 2a, related to the aerogel at 10 % w/w), indicating that, probably, the chosen exchanging time was not sufficient for the formation of the alcogel, and, then, for the attainment of a nanostructured aerogel. Choosing a higher time for the solvent exchange (24 h for each step), the potato starch aerogel, at all the tested concentrations, showed a fibrous nanostructure (as clear from the FESEM image in Figure 2b).

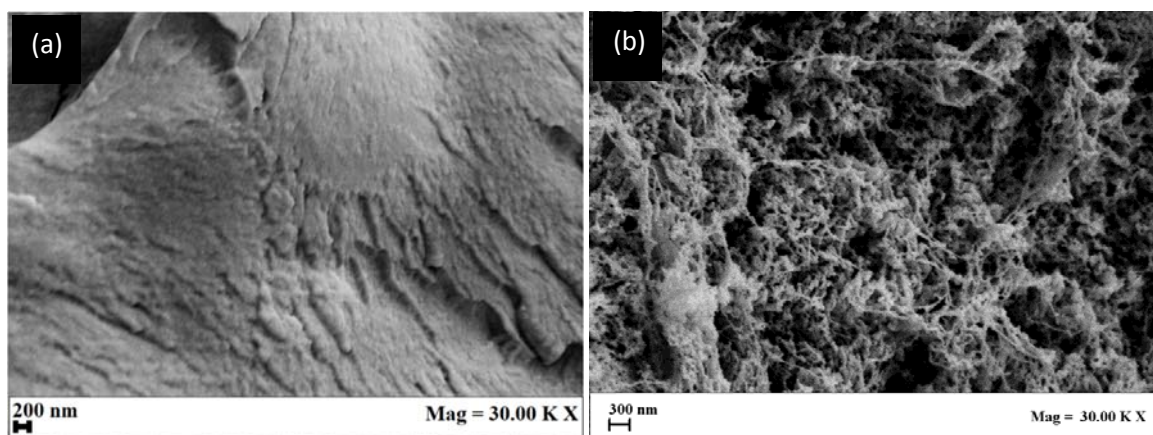


Figure 2: FESEM images of potato starch aerogels obtained with a supercritical gel drying at 200 bar, 45 °C, 10 % w/w: (a) solvent exchanging time equal to 2 h; (b) solvent exchanging time equal to 24 h

3.2 Wheat starch

Wheat starch alcogels were prepared following the procedures described before, varying the concentration of starch in water (5-15 %) and the time for each step of the solvent exchanging (2 h and 24 h). Then, they were dried by scCO_2 to obtain the corresponding aerogels. For that kind of starch, the solvent exchanging time had

no significant effect (2 h for each step were sufficient) on the morphology of the obtained aerogel; on the contrary, the aerogels prepared at different concentrations were very different from the morphological point of view. Indeed, in correspondence of a concentration of 5 % w/w, the aerogel was constituted by microparticles with a nanoporous structure, as evidenced in Figure 3a, whereas, in correspondence of higher concentrations, the wheat starch aerogel showed a lenticular structure with closed pores (the surface appeared continuous also at high enlargements), as shown in Figure 3b.

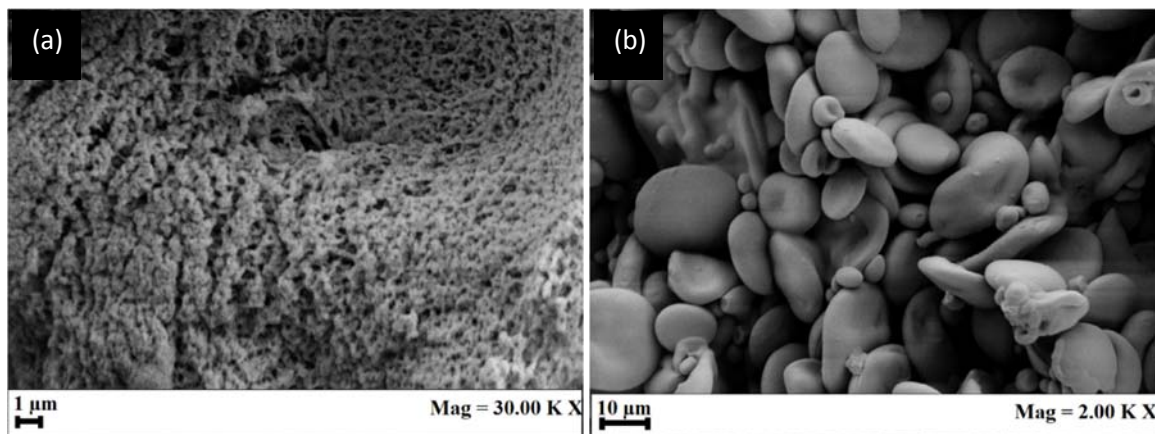


Figure 3: FESEM images of wheat starch aerogels obtained with a supercritical gel drying at 200 bar, 45 °C at different starting concentrations: (a) 5 % w/w; (b) 10 % w/w

3.3 Maize starch

Maize starch alcogels were prepared following the procedures described before, varying the concentration of starch in water (5-15 %) and the time for each step of the solvent exchanging (2 h and 24 h). Then, they were dried by scCO_2 to obtain the corresponding aerogels. As in the case of wheat starch, the solvent exchanging time had no significant effect (2 h for each exchanging step was a sufficient time) on the morphology of the obtained aerogel; moreover, as in the case of potato starch, the aerogels prepared at different concentrations were very similar to each other from the morphological point of view. Indeed, at all the starting concentrations in the range 5 – 15 % w/w, the aerogel was constituted by microparticles with a nanoporous structure, as evidenced in Figures 4a and 4b, where maize starch aerogels obtained at different starting concentrations are reported.

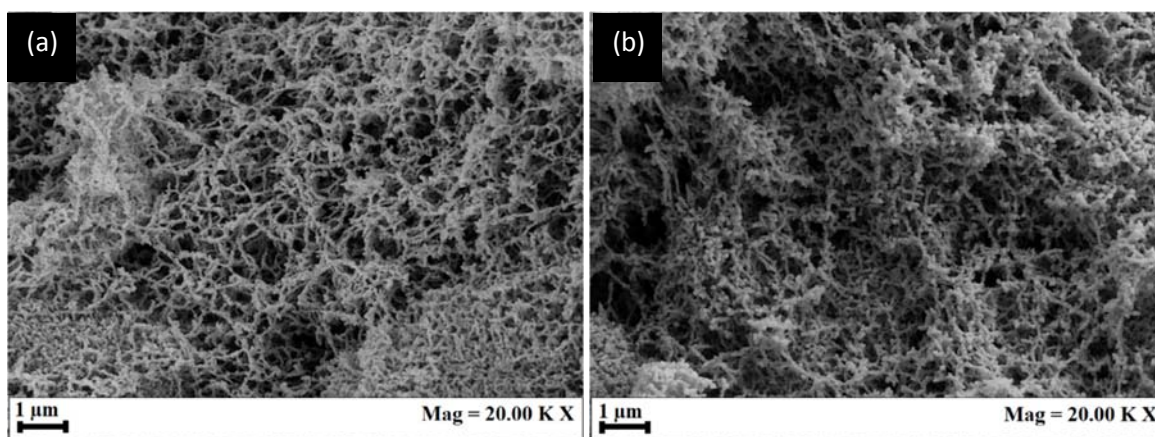


Figure 4: FESEM images of maize starch aerogels obtained with a supercritical gel drying at 200 bar, 45 °C at different starting concentrations: (a) 5 % w/w; (b) 15 % w/w

3.4 Analyses

DSC measurements of the starch aerogels were performed. The curve related to the maize starch aerogel is reported in Figure 5. A broad endothermic event with a peak at 82 °C is attributed to the gelatinization peak temperature.

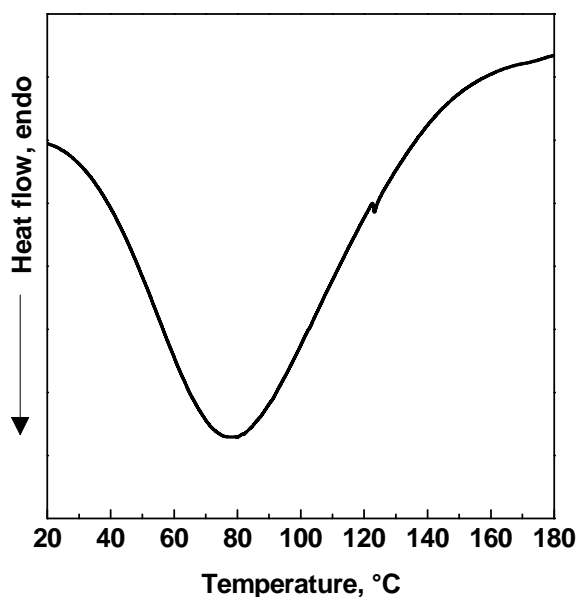


Figure 5: Differential scanning calorimetry thermogram for maize starch aerogel after baseline subtraction. Endo indicates endothermic flow

The residual moisture of the starch aerogels was determined by measuring the mass loss resulting from heating at 110 °C the aerogel and was equal to 14 % w/w. The particle density used for calculation of porosity is 1.5 g/cm³, according to Marousis and Saravacos (1990). The surface area of the maize starch aerogel using the BET method was calculated and equal to 62 m²/g. This value is in accordance with literature (Mehling et al., 2009). Indeed, for the preparation of the aerogels in our experimentations, a retrogradation time equal to 3 days was chosen, whereas Mehling et al. (2009) made surface area measurements using the BET method extending retrogradation times from 2 to 6 days and obtaining an increase of the surface area from 52 to 83 m²/g.

4. Conclusions

ScCO₂ drying process was used to produce biopolymeric aerogels starting from hydrogels; in particular, different source starches were processed, demonstrating that the source, the time of the solvent exchanging and the concentration of the starting solution have an effect on the morphology and on the porosity of the obtained aerogels. The nanoporous morphology obtained, at specific conditions, confirmed the potentiality of these structures as drugs delivery devices.

References

- Caputo G., De Marco I., Reverchon E., 2010, Silica aerogel–metal composites produced by supercritical adsorption, *J. Supercritical Fluids*, 54, 243–249.
- Caputo G., Scognamiglio M., De Marco I., 2012, Nimesulide adsorbed on silica aerogel using supercritical carbon dioxide, *Chemical engineering research and design*, 90, 1082–1089.
- Cardea S., Baldino L., De Marco I., Pisanti P., Reverchon E., 2013, Supercritical gel drying of polymeric hydrogels for tissue engineering applications, *Chemical Engineering Transactions*, 32, 1123–1128.
- De Marco I., Cardea S., Reverchon E., 2013, Polymer Micronization using Batch Supercritical Antisolvent Process, *Chemical Engineering Transactions*, 32, 2185–2190.
- García-González C.A., Alnaief M., Smirnova I., 2011, Polysaccharide–based aerogels – Promising biodegradable carriers for drug delivery systems, *Carbohydrate Polymers*, 86, 1425–1438.
- García-González C.A., Smirnova, I., 2013, Use of supercritical fluid technology for the production of tailor-made aerogel particles for delivery systems, *J. Supercritical Fluids*, 79, 152–158.

- Glenn G.M., Stern D.J., 1999, Starch-based microcellular foams, US Patent 5,958,589, url: www.google.com/patents/US5958589.
- Hakuta Y., Hayashi H., Arai K., 2003, Fine particle formation using supercritical fluids, *Current Opinion in Solid State & Materials Science*, 7 (4–5), 341–351.
- Hezave A.Z., Esmailzadeh F., 2010, Micronization of drug particles via RESS process, *J. Supercritical Fluids*, 52, 84–98.
- Ho M.H., Kuo P.Y., Hsieh H.J., Hsien T.Y., Hou L.T., Lai J.Y., Wang D.M., 2004, Preparation of porous scaffolds by using freeze-extraction and freeze-gelation methods, *Biomaterials*, 25, 129–138.
- Jin R., Moreira Teixeira L.S., Dijkstra P.J., Karperien M., Zhong Z., Feijen J., 2008, Fast in-situ formation of dextran-tyramine hydrogels for in vitro chondrocyte culturing, *J. Controlled Release*, 132, 24–26.
- Malafaya P., Stappers F., Reis R., 2006, Starch-based microspheres produced by emulsion crosslinking with a potential media dependent responsive behavior to be used as drug delivery carriers, *Journal of Materials Science: Materials in Medicine*, 17, 371–377.
- Marousis S.N., Saravacos G.D., 1990, Density and porosity in drying starch materials, *Journal of Food Science*, 55, 1367–1372.
- Marra F., De Marco I., Reverchon E., 2012, Numerical analysis of the characteristic times controlling supercritical antisolvent micronization, *Chem. Eng. Sci.*, 71, 39–45.
- Mehling T., Smirnova I., Guenther U., Neubert R.H.H., 2009, Polysaccharide-based aerogels as drug carriers, *J. Non-Crystalline Solids*, 355, 2472–2479.
- Prosapio V., Reverchon E., De Marco I., 2014, Antisolvent micronization of BSA using supercritical mixtures carbon dioxide + organic solvent, *J. Supercritical Fluids*, 94, 189–197.
- Quirk R.A., France R.M., Shakesheff K.M., Howdle S.M., 2005, Supercritical fluid technologies and tissue engineering scaffolds, *Current Opinion in Solid State & Materials Science*, 8, 313–321.
- Reverchon E., Antonacci A., 2007, Polymer microparticles production by supercritical assisted atomization, *J. Supercritical Fluids*, 39(3) 444–452.
- Reverchon E., Cardea S., Rapuano C., 2008, A New supercritical fluid based process to Produce Scaffolds for tissue replacement, *J. Supercritical Fluids*, 45, 365–373.
- Reverchon E., De Marco I., 2011, Mechanisms controlling supercritical antisolvent precipitate morphology, *Chemical Engineering Journal*, 169, 358–370.
- Shariati A., Peters C.J., 2003, Recent developments in particle design using supercritical fluids, *Current Opinion in Solid State and Materials Science*, 7, 371–383.
- Smirnova I., Mamic J., Arlt W., 2003, Adsorption of drugs on silica aerogels, *Langmuir*, 19, 8521–8525.
- Torino E., De Marco I., Reverchon E., 2010, Organic nanoparticles recovery in super-critical antisolvent precipitation, *J. Supercritical Fluids*, 55 (1), 300–306.