Carbohydrate Polymers 88 (2012) 1378–1386

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Preparation of tailor-made starch-based aerogel microspheres by the emulsion-gelation method

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ARTICLE INFO

Article history:
Received 24 November 2011
Received in revised form 31 January 2012
Accepted 13 February 2012
Available online 22 February 2012

Keywords:
Starch
Aerogel
Supercritical drying
Emulsion-gelation
Chemical carrier matrix

ABSTRACT

The inherent biocompatibility and biodegradability of starch, a natural polysaccharide-based product, allows its use in the form of microspheres as a chemical carrier for life science applications. However, current methods of preparation of starch microspheres utilize chemical crosslinkers and drying methods (air drying, freeze drying) that leads to problems of degradability of the matrix and low specific surface areas and chemical loading capacities. In this work, corn starch aerogel microspheres, a special class of nanoporous materials, were prepared by the combination of an emulsion-gelation method and supercritical drying without the use of chemical crosslinkers. Effects of gelation temperature (368, 393 and 413 K), oil-to-aqueous starch solution ratio (1:1, 2:1, 3:1) and surfactant content (3, 6 and 10% (w/w)) on the textural and morphological properties of the aerogel material were studied. The obtained starch aerogels were characterized using nitrogen adsorption–desorption measurements, helium pycnometry, CHN elemental analyses, thermogravimetry and scanning electron microscopy. Spherical starch aerogel microspheres with tailor-made specific surface areas (34–120 $m^2\,g^{-1}$ range) and particle sizes (215–1226 μm diameter range) were obtained. Aerogel textural properties were mainly influenced by the gelation temperature used, whereas the particle morphology depended on the three processing parameters studied. High specific chemical loading capacity ($1.1 \times 10^{-3}\,g\,m^{-2}$) of the aerogel microspheres was obtained using ketoprofen as a model compound.

1. Introduction

Microparticles made from natural products (e.g., polysaccharides), in the form of microspheres or microcapsules, have been proposed as advantageous delivery carriers for the controlled release of active compounds, agrochemicals and food bioactives for life sciences applications (Elfstrand, Eliasson, & Wahlgren, 2009; Li et al., 2009; Malafaya, Stappers, & Reis, 2006; Wing, Carr, Trimnell, & Doane, 1991). These carriers can effectively protect the entrapped substance against environmental degradation (e.g., pH, temperature, humidity, enzymes, microorganisms) (Dumitriu, 2005; Müller, Mäder, & Gohla, 2000; Smith & Williams, 2006). The microparticulate carrier can be designed for use in various routes of administration as well as for the controlled and targeted release of active chemicals. This customized performance of the carrier leads to an enhanced efficacy of delivery, reduced toxicity, and improved customer acceptance.

Starch is an abundant, edible, low toxic and low-cost polysaccharide found in the leaves, seeds and tubers of many vegetables (e.g., potato, corn, pea, wheat, tapioca) in the form of granules. The good biodegradability and stability of starch and its versatility in processing (Doane, 1992; Duarte, Mano, & Reis, 2009; Dumitriu, 2005; Wing et al., 1991) makes it a promising delivery carrier for drug, biomedical, agriculture and food applications. The substance to be entrapped can be physically (adsorption) or chemically attached to the starch matrix (Miao, Li, Deng, Wang, & Liu, 2010; Yang, Wei, Sun, & Wan, 2010). For pharmaceutical purposes, starch microspheres have been reported to be used via the nasal, parenteral, oral administration routes for magnetic resonance imaging, chemotherapy for liver cancer or drug delivery among others (Fang et al., 2008; Ishida et al., 2008; Kim et al., 2003; Mundargi, Shelke, Rokhade, Patil, & Aminabhavi, 2008). Starch microspheres are also proposed for tissue engineering applications by simultaneous injection with the scaffold at the site of regeneration and for encapsulation of living cells (Malafaya et al., 2006). In agriculture, starch microspheres meet the market demand for easy-to-handle carriers allowing the effective slow-release of agrochemicals, thus reducing the amount of chemical to be applied, the frequency of application and the environmental impact (Glenn et al., 2010). For the food industry, starch is used in edible films to coat food and also

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doi:10.1016/j.carbpol.2012.02.023

to mimic pharmaceutical applications for the controlled release of food bioactives (e.g., vitamins, peptides, probiotics, antioxidants) (Li et al., 2009).

Production of starch microparticles obtained by grinding (Wing, Maiti, & Doane, 1988) or in the form of microspheres by spray drying and by emulsion cross-linking techniques (water-in-oil (w/o) or water-in-water (w/w)) have been reported in the literature (Li et al., 2009; Malafaya et al., 2006; Mao, Chen, Wei, Liu, & Bi, 2004). Starch microspheres are usually produced using chemical crosslinkers, but the use of these chemicals increase the end material costs. As well, the bonds formed in the chemically crosslinked starch matrices are often irreversible at human body temperatures thus hindering the degradation of the entire microparticle. The production of these microparticles without cross-linkers can be achieved by taking advantage of the starch ability to gel upon heating (i.e., thermal gelation (Glenn et al., 2008; Nelles, Dewar, van der Merwe, & Taylor, 2003)). The preparation of starch microspheres by thermal gelation using spray drying was reported in the literature (Glenn et al., 2010). In this work, the preparation of starch microspheres using a water-in-oil emulsion-gelation mechanism is developed.

The textural properties of the dried microspheres (i.e., density, pore size, surface area) determine the adsorption behavior of chemicals in the matrix as well as the maximum adsorption capacity of the microspheres. In general, high surface area and pore volume of the microspheres favor their loading capacity (García-González, Alnaief, & Smirnova, 2011). The drying of the starch microspheres is regarded as the most critical step in obtaining starch microparticles in the dry form while preserving the original nanoporous structure of the wet gel. Air drying of starch gels are not able to preserve the gel structure leading to xerogels with significant pore collapse, shrinkage of the gel structure, formation of cracks and appearance of macropores (Glenn et al., 2010; Marousis & Saravacos, 1990). Freeze drying of starch gels produces highly porous structures by the removal of water vapor form the frozen gel, but was found to have a destructive effect on the structure of the starch (Elfstrand et al., 2009). The occurring sublimation of the ice crystals upon freeze-drying may result in a ballooning of the organic gel structure, leading to the formation of macropores and the decrease of the specific surface area (Franks, 1998). Moreover, local variations in nucleation and growth rates of ice crystals between the inner and outer part of the gel may happen during the freeze drying process resulting in different pore structures in the cryogel as a function of depth. In contrast, supercritical drying of wet starch gels create aerogels, which are dry lightweight materials with outstanding surface area and open porosity (Akimov, 2003; Hüsing & Schubert, 1998). This drying process avoids the pore collapse phenomenon and preserves the nanoporous texture of the wet material. The superior porous properties of aerogels make them suitable for loading with large volumes of chemical compounds (Gorle, Smirnova, & McHugh, 2009; Miao, Jing-xiao, Fei, Ji-hong, & Shuang, 2006; Rolison, 2003; Smirnova, Suttiruengwong, & Arlt, 2004; Smirnova, Türk, Wischumerski, & Wahl, 2005). The use of supercritical fluids for the drying of starch gels was already reported for cylinders (Mehling, Smirnova, Guenther, & Neubert, 2009) and this method is presented in this work for starch microparticles for the first time.

In this work, the ability of starch to gel upon thermal treatments (i.e., thermotropic gel) was exploited to combine sol–gel, emulsion and supercritical drying technologies to obtain aerogel spherical particles from starch with high internal surface area for potential chemical delivery applications. Influence of processing parameters (temperature, oil-to-starch aqueous solution phase ratio, use of surfactants) on both particle morphology and textural properties were studied. Finally, the potential use of starch aerogel microspheres for life science applications was assessed regarding the loading capacity of chemicals using ketoprofen as a model compound.

2. Experimental methods

2.1. Reagents

Native corn starch (Starch Amylo N-460; amylose content: 52.6%; labeled as raw) was provided by Roquette. Ethanol (99.8% purity) and vegetable (canola) oil were obtained from Omnilab and domestic shops, respectively. Deionized water was used in all gelation experiments. Glyceryl monostearate (GMS, technical grade) and ketoprofen were provided by Lambent Technologies and Chemische Fabrik Kreussler & Co. GmbH, respectively. CO_2 (>99.9 mol% purity) was supplied by AGA Gas GmbH.

2.2. Processing

2.2.1. Preparation of starch gel microspheres

The emulsion-gelation method was used to obtain gel microspheres and was adapted from the emulsion crosslinking method reported in the literature for other types of gel (Alnaief, Alzaitoun, García-González, & Smirnova, 2011; Alnaief & Smirnova, 2011; Mayer, Kong, Pekala, & Kaschmitter, 1996) (Fig. 1). Oil:starch solution emulsions with 1:1 (2:1 and 3:1) volume phase ratios were prepared by mixing 30 g of a 15% (w/w) corn starch dispersion in water with the corresponding amount of vegetable oil. In some cases, surfactant was also added to the emulsion with varying concentrations (3%, 6% and 10% (w/w) relative to the starch solution phase and labeled as 3% GMS, 6% GMS and 10% GMS, respectively). The resulting emulsion was then heated up to different temperatures (368, 393 and 413 K) and pressurized at 0.1–0.2 MPa in a closed autoclave (Berghof RHS 295) equipped with magnetic stirring (300 rpm). Once the desired temperature and pressure were reached, these conditions were kept constant for 20 min. Then, the pressure of the autoclave was released and the temperature of the emulsion lowered up to 318 K using an ice bath (cooling rate: 3 K min^{-1}) under agitation (marine type; d = 40 mm; stirring: 1400 rpm). After centrifugation (4500 rpm, 277 K, 5 min), particles were separated from the oil phase, soaked in ethanol and placed in the refrigerator (277 K) for retrogradation for 48 h. After retrogradation, starch particles were transferred to a fresh ethanol solution (i.e., second solvent exchange).

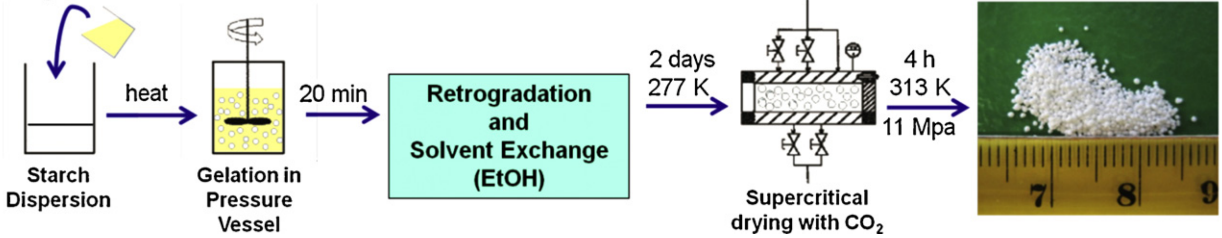

Fig. 1. Diagram of the processing method used for the preparation of starch aerogel microspheres.

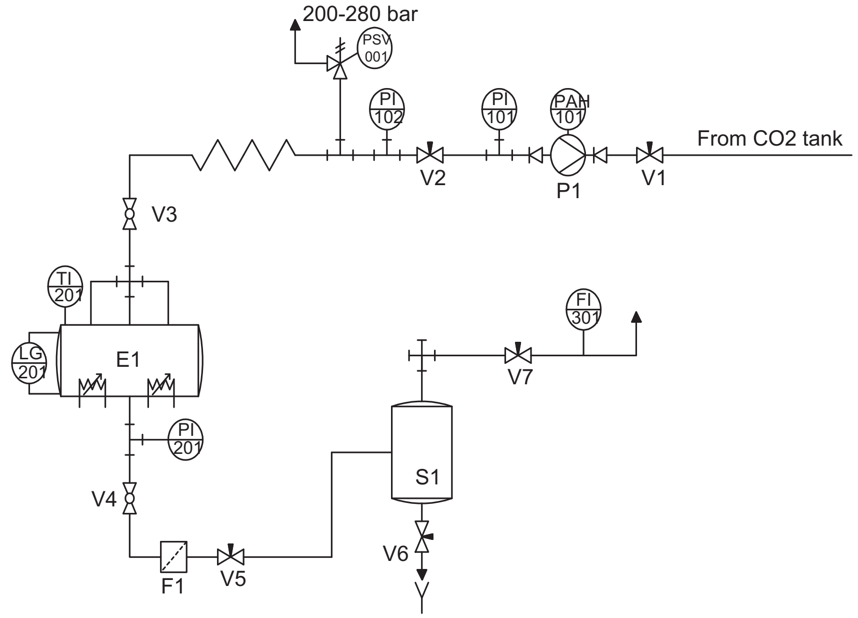

Fig. 2. Process flow diagram of the equipment used for the supercritical drying of starch aerogels. Tags: V1–V7 = valves; P1 = compressor; E1 = autoclave; S1 = separator; F1 = filter; PAH-101 = high-level pressure alarm; PI-xxx = pressure gauges; PSV-001 = safety valve; TI-201 = thermocouple; LG-201 = sapphire window; FI-301 = flow meter.

2.2.2. *Supercritical drying of starch gel microspheres*

The resulting starch alcogels were dried by extraction of the solvent with a continuous flow of supercritical carbon dioxide ($scCO_2$) using the equipment sketched in the process flow diagram (Fig. 2). In a typical experiment, the starch alcogels were enclosed in cartridges made of filter paper and placed into a 250-ml autoclave (E1 in Fig. 2). Samples were loaded into the autoclave immersed in ethanol to prevent shrinkage due to evaporation of ethanol in the alcogel network before exposure to $scCO_2$. The autoclave was heated to 313 K by a thin electrical band heater. Once the temperature in the autoclave was constant, CO_2 was fed by a high-pressure diaphragm compressor (P1, Whitey LC10) to the autoclave containing the gels until the desired working pressure (11.0–12.0 MPa) was reached. Then, the outlet $scCO_2$ flow from the autoclave was started by opening valve V4 and the flow rate (2–4 N l min^{-1}) was regulated by the micrometering valve V5. The starch microspherical alcogels were dried under these operating conditions for 4 h to assure a complete removal of the solvent. Finally, the pressure was released (depressurization) slowly within 30–60 min at constant temperature (313 K) until atmospheric pressure was reached.

2.2.3. *Drug adsorption of starch aerogels*

The starch aerogels (1 g) and the ketoprofen (1 g) were weighed and enclosed separately in cartridges made of filter paper and placed into a 40-ml autoclave. After closing the autoclave, it was preheated at 323 K and then pressurized with CO_2 (20.0 MPa). Under agitation at 300 rpm, the drug was dissolved in $scCO_2$ and adsorbed on the aerogel matrix. After 24 h, the system was depressurized and the drug-loaded aerogel microspheres were recovered for further characterization.

2.3. *Physicochemical characterization of the starch aerogels*

Micrographs of the samples were recorded in a scanning electron microscope (SEM, Leo Zeiss 1530). All samples were gold-sputtered (10 nm thickness) prior to imaging in order to minimize charging and improve the image quality (contrast). Textural properties of the starch aerogels were determined by low-temperature N_2 adsorption–desorption analysis (Nova 3000e). Prior to measurements, samples were dried under vacuum (<1 mPa) at 353 K for 20 h. Specific surface area (A_{BET}) was determined by the BET (Brunauer–Emmett–Teller) method. Pore volume (V_p) and mean pore diameter (d_p) were estimated using the BJH (Barrett–Joyner–Halenda) method. The particle skeletal density (ρ_{skel}) of the starch-based materials was determined from five replicates (standard deviation <1%) by gas displacement measurements using a helium-pycnometer (Micromeritics Multivolume Pycnometer 1305) at 298 K and 0.13 MPa. CHN analyses of the samples were performed in duplicate with a Vario MICRO cube (Elementar Analysensysteme) elemental analyzer. The quantification of the water content of samples was evaluated thermogravimetrically using a TGA Netzsch TG 209 F1 instrument. The degradation processes were investigated by heating 8–10 mg of the aerogel sample contained in alumina analysis crucibles in a thermal balance. Measurements were performed under a nitrogen atmosphere with a heating rate of 10 K min^{-1}. Particle size distribution of the wet microspheres dispersed in ethanol was measured using laser diffraction spectrometer (Beckman Coulter LS1332) and with a similar obscuration value. Mean particle diameter (D_m) and Sauter mean diameter ($D(3,2)$) were calculated based on the measured particle size distributions. Drug loading content of ketoprofen-loaded aerogels was photometrically quantified in triplicate (UV–Vis ThermoScientific Evolution 300, $\lambda = 254$ nm). For drug extraction from the aerogel matrix, ketoprofen-loaded aerogels are milled and dispersed in ethanol. After 30 min of sonication, the dispersions are centrifuged and the supernatant solution analyzed.

3. Results and discussion

3.1. *Preparation of starch aerogel microspheres*

The original appearance of the native corn starch granules was of lenticular to irregular shape with a smooth, solid and non porous surface (Fig. A in Supplementary Information). Starch undergoes gelation in a three-step thermally assisted hydration–plasticization of the polysaccharide network. In the first step, *swelling* takes place by adsorption of water in the hydrophilic starch granules (Wootton & Bamunuarachchi, 1979) leading to an increase in diameter of more than 200% with respect to the original size of the granule

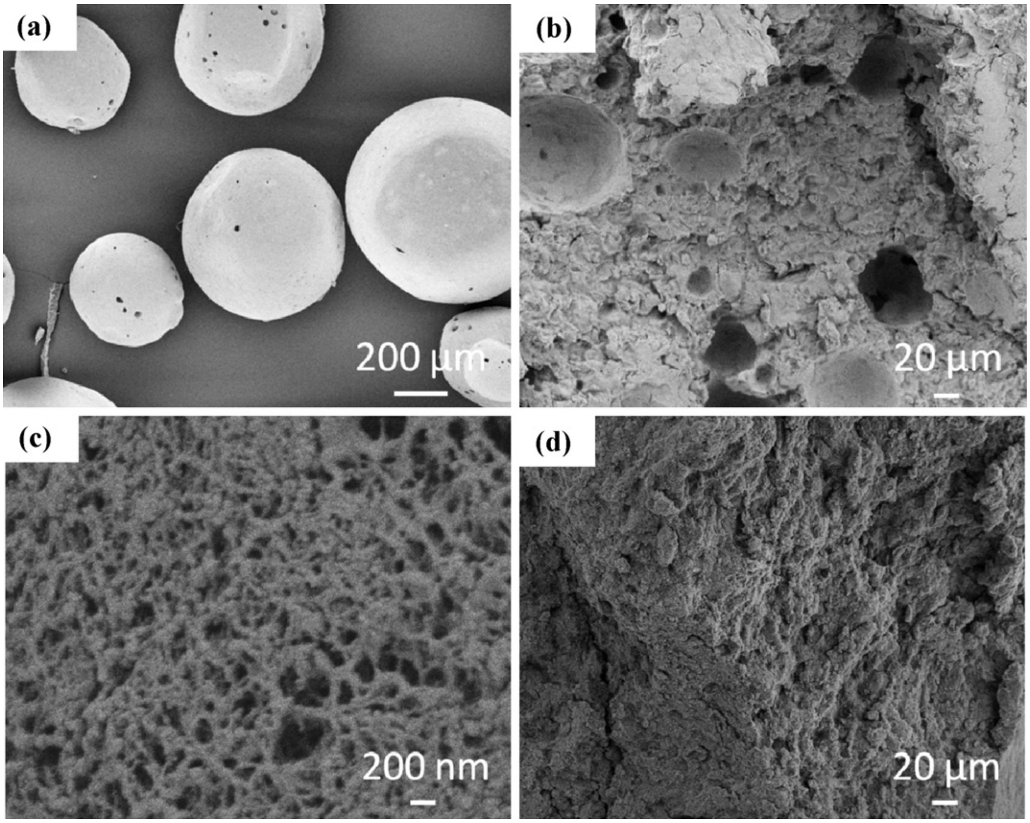

Fig. 3. Preparation of starch aerogels from water-in-oil emulsions (o/w phase ratio = 2:1) by mixing both phases before gelation of the starch aqueous solution at 393 K. External (a) and internal (b: longitudinal cut of particles using a scalpel) appearance and nanostructure (c) of the starch aerogel particles obtained after supercritical drying. (d) Cut of a starch aerogel monolith processed at the same conditions without oil addition.

(Atkin, Abeysekera, Cheng, & Robards, 1998). Then, *gelatinization* takes place after starch is dissolved by heating, leading to leaching of amylose molecules, irreversible physical changes and the destruction of the granule structure. Finally, in the so-called *retrogradation* step, the starch hydrogel structure is formed upon cooling and aging, followed by the reorganization and the partial recrystallization of the polysaccharide structure. Amylose content and gelatinization temperature are the main process parameters influencing the texture of the gel formed (Barker, 2010; White, Budarin, & Clark, 2008).

The thermotropic origin of starch gels was taken into account in this work to choose the proper strategy to obtain starch microspheres from water-in-oil emulsions. The criterion of eliminating the use of any toxic organic solvent was adopted to fulfill health/environmental considerations in regards of their application to the biomedical sciences. The oil phase was added to the starch aqueous dispersion in the first step of the gelation process before heating the mixture up to 393 K. A rapid cooling of the starch gels was performed up to 277 K to favor crystal nucleation with respect to crystal growth and recrystallization, thus leading to higher surfaces areas in the resulting aerogel (Mehling et al., 2009; Walter, 1998). Samples were stored at low temperature (277 K) during retrogradation for 48 h to promote the sphericity of the microparticles and an amorphous starch structure with few, non-perfect crystallites (Durrani & Donald, 1995; Elfstrand et al., 2009). The high critical point of water ($P_c = 22.1$ MPa; $T_c = 647.1$ K) hampers the direct supercritical drying of the hydrogel because starch will degrade at the drying conditions needed: $P > P_c$ and $T > T_c$. Moreover, the low solubility of water in scCO$_2$ hinders its extraction using the milder conditions of scCO$_2$-assisted drying technique (for CO$_2$, $P_c = 7.38$ MPa; $T_c = 304.1$ K). Therefore, water solvent in the starch hydrogel was replaced by ethanol through solvent exchange before supercritical drying. Ethanol was chosen for its high solubility in scCO$_2$

Fig. 4. Visual appearance of supercritically dried starch aerogel particles obtained from water-in-oil emulsions (o/w ratio = 2:1) gelled at 393 K and then stirred upon cooling at: (a) 600 rpm, (b) 1400 rpm, and (c) 1400 rpm with 3% (w/w) of surfactant.

and miscibility with water together with being a poor solvent for starch.

Fig. 3a–c shows the resulting starch aerogel microspheres obtained after supercritical drying. Spherical particles of starch in the range of 200–400 μm with a nanoporous texture characterized by high specific surface area and total mesopore volume were obtained (Table 1). Stirring rate upon cooling played an important role in the particle size of the aerogel: the higher the stirring rate was, the lower the particle size obtained (Fig. 4). The average pore diameter (15–20 nm) of the obtained starch aerogels was in the range of the average distance between crystalline layers of native starch (9 nm) (Vermeylen, Goderis, Reynaers, & Delcour, 2004), likely indicating that the origin of the pores of the aerogel was the reassociation of the starch components in an expanded form (Budarin et al., 2006). Besides the mesoporous structure, certain non-interconnected macropores were observed likely resulting from some oil droplets entrapped inside the wet gel microspheres and removed upon supercritical drying. They do not seem to be remnants of swollen starch granules (ghosts) because the pores are empty with no presence of a porous network inside, and there is also no presence of granule walls (Durrani & Donald, 1995; Elfstrand et al., 2009; Glenn et al., 2008). It has also been reported that the heating of starch aqueous slurries above 393 K leads to complete dissolution of the ghosts (Glenn et al., 2008). Macropores cannot be related to the drying process since no radial channels from the surface to the center of the particles or irregular cracks were observed as reported for the air drying of polysaccharide gels (Di Renzo et al., 2005; Marousis & Saravacos, 1990). To confirm this hypothesis, the same experiment with the absence of the oil phase was carried out using the same volume of starch solution as the total volume of the emulsion. No big pores were present in the resulting starch aerogel monolith (Fig. 3d). Therefore, under the conditions used to process the starch, starch granules completely solubilized and produced a porous starch matrix devoid of any granule remnant. For the sake of comparison, values on textural properties of other starch gel microspheres are unfortunately not available in the literature. Glenn et al. (2010) reported some SEM pictures of starch microspheres obtained by spraying followed by drying under a N_2 atmosphere presenting a markedly macroporous structure. Elfstrand, Eliasson, Jönsson, Reslow, and Wahlgren (2006) reported micrographs of starch particles obtained by freeze drying of an aqueous two-phase system with a rough mesoporous surface pattern and there is no information available about the inner textural properties.

3.2. *Study of processing parameters*

3.2.1. *Effect of gelation temperature*

The structure of starch aerogel microspheres depends directly on the gelation temperature in which the wet gel is prepared. The aqueous starch solution of the dispersed phase in the water-in-oil emulsion becomes viscous upon heating due to the sequence: swelling of the granules → leaching of amylose molecules → burst and collapse of the granules, leading to a concomitant drop in viscosity (Glenn et al., 2008). After 20 min of heating at 368 K, the corn starch granules were found to not be completely burst and dissolved, as noticed by the swollen granules of 20–30 μm obtained in the form of agglomerates (Fig. 5a, 434 μm of mean particle size). A mesh of fibrous strands is observed over the surface of the aerogel particle (Fig. 5b). These strands result from the soluble starch fraction (mainly amylose) leached from the interior of the granules (Nelles et al., 2003). The surface deposition of this leached component is likely responsible for the observed surface area in the material (Table 1).

Higher temperatures were effective in getting disaggregated starch aerogel microspheres without traces of any remaining native

Table 1
Physicochemical characterization of starch aerogel microspheres produced from water-in-oil emulsions (2:1 oil:water volume ratio).

Gelation temperature	CHN analysis				He-pycnometer	TGA	N_2 adsorption–desorption analysis		Particle size of the wet gel	
	% C	% H	% N	C/H ratio	ρ_{skel}, g cm^{-3}	Weight loss at 298–473 K, wt.%	A_{BET}, m^2 g^{-1}	V_p, cm^3 g^{-1}	D_m, μm	$D(3,2)$, μm
Raw	39.72 ± 0.08	6.60 ± 0.04	1.33 ± 0.01	6.0	1.49 ± 0.01	8.4	<5	–	–	–
368 K	–	–	–	–	1.38 ± 0.06	–	66 ± 3	0.32 ± 0.02	424 ± 21	64 ± 2
393 K	42.20 ± 0.38	6.76 ± 0.07	1.23 ± 0.04	6.2	1.35 ± 0.03	5.8	112 ± 6	0.37 ± 0.02	1277 ± 3	1133 ± 3
413 K	50.64 ± 0.13	7.92 ± 0.03	1.14 ± 0.13	6.4	1.17 ± 0.01	2.3	40 ± 2	0.18 ± 0.01	417 ± 1	84 ± 1
393 K, 3% GMS	46.31 ± 0.25	7.2 ± 0.02	1.24 ± 0.07	6.4	1.34 ± 0.01	2.8	103 ± 5	0.32 ± 0.02	519 ± 3	110 ± 1
393 K[a]	–	–	–	–	1.45 ± 0.05	7.7	247 ± 12	0.84 ± 0.04	–	–
368 K[b]	–	–	–	–	–	1.8	90 ± 18	0.37 ± 0.05	–	–

[a] Data for corn starch aerogel cylinder processed using starch solutions of the same total volume as for the starch aerogel particles followed by supercritical drying.

[b] Data for high-amylose content corn starch aerogel cylinder reported in the literature (Mehling et al., 2009).

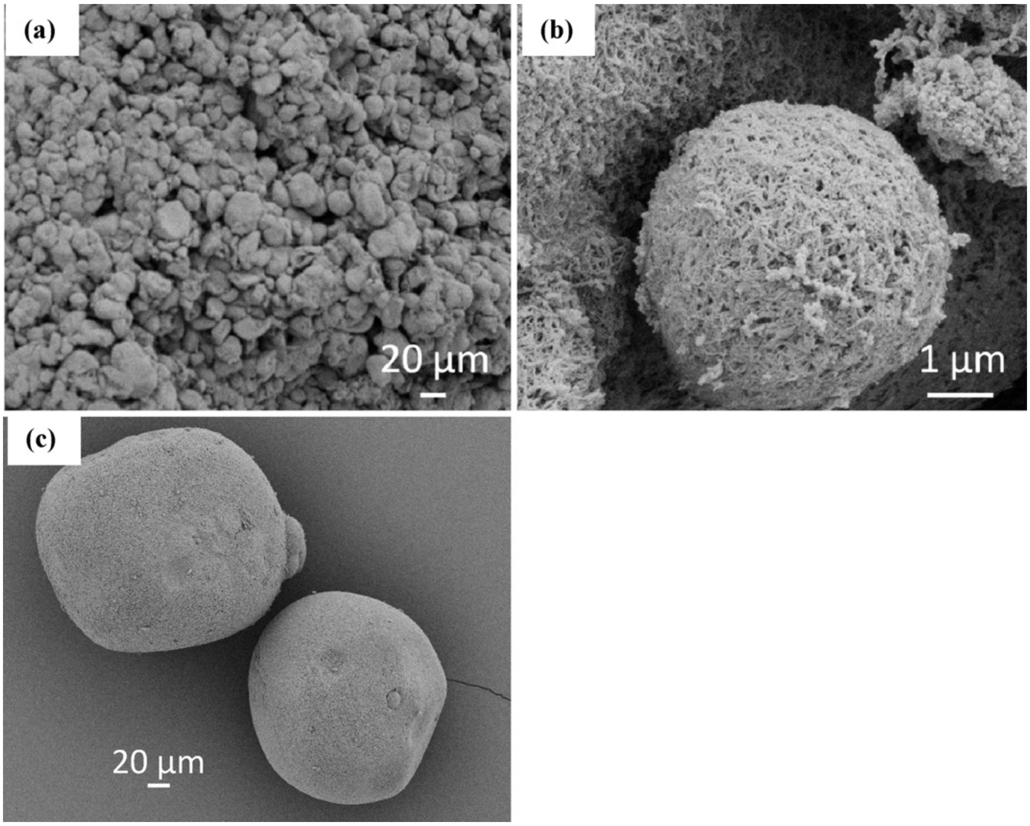

Fig. 5. SEM micrographs of starch aerogel microspheres processed at the gelation temperatures of (a and b) 368 K and (c) 413 K. Remnants of swollen starch granules were still evident when the low processing temperature was used.

starch granule (Figs. 3a, b and 5c). The textural properties of starch aerogels were improved up to a certain temperature (393 K), beyond which this trend was reversed (Table 1). This decrease in the textural properties above a certain threshold value has been related to the start of the starch depolymerization through α-(1,6) hydrolysis (White et al., 2008) and the removal of structural water upon heating. As a result, the gel strength decreases with more intensive heating treatments (Glenn et al., 2008). A greater compression of the starch aerogel matrices was observed at 413 K leading to compacted low surface area particles with lower particle size (Table 1). The decrease in viscosity of the starch gel with temperature facilitating the breaking up of the dispersed phase may also contribute to the formation of smaller particles at 413 K than at 393 K. The different origin of the textural properties of the aerogel processed at 368 K (amylose deposition on the granule surface) and at 393 K (destruction of the starch granule structure followed by the reorganization of the polysaccharide structure upon cooling) may explain the differences in the textural properties observed between these microspherical samples (Table 1).

The He-pycnometry measurements of the starch particles showed differences in the skeletal densities of the material with increasing gelation temperature. The skeletal density of the native corn starch was 1.49 g cm^{-3}, whereas starch aerogel microspheres processed at 368, 393 and 413 K had skeletal densities of 1.38, 1.35 and 1.17 g cm^{-3}, respectively. The skeletal density strongly depends on the moisture content of the starch sample. Sorbed water in starch leads to an increase in the skeletal density of the particle up to a certain moisture content (0.15 kg water/kg dry starch) where this trend is reversed and density of sorbed water approaches to that of liquid water (Marousis & Saravacos, 1990). Therefore, the obtained results indicate a reduction in skeletal density of the starch aerogel structure due to dehydration, with this reduction being proportional to the gelation temperature used.

CHN analyses (Table 1) confirmed that structural compaction of starch aerogels is influenced by water removal. Results showed an increase in the carbon content and the carbon-to-hydrogen ratio with gelation temperature likely due to the increase of the sample content in the carbohydrate backbone with respect to structural water. Thermogravimetric analyses were also carried out to study the thermal weight losses between 298 and 473 K of starch aerogel samples, arising from the removal of physisorbed water (Mehling et al., 2009). Maximum weight loss was observed for the raw starch (8.4 wt.%) whereas it decreased with increasing gelation temperature used for starch aerogel particles processing (Table 1). The main reason behind this increased dehydration with temperature is that, during starch gel processing at 393 and 413 K, the operating pressure (0.1–0.2 MPa) is below the water vapor pressure at these temperatures (0.20 and 0.36 MPa for 393 and 413 K, respectively) (Wagner & Pruss, 1993), and water removal due to evaporation is likely to occur. The thermal weight loss between 298 and 473 K of a supercritically dried starch aerogel monolith, processed in the same closed vessel as for the processing of starch particles, at a gelation temperature of 393 K and using the same volume of starch solution as the total volume of the emulsion used for the particles was significantly higher (Table 1). The higher water content in the vessel for the gelation of starch gel monoliths leads to less water evaporation than for the case of particles and, as a result, aerogels with higher structural water content and better textural properties were obtained. Low water content and lower textural properties were reported in the literature for starch aerogel cylinders (Table 1) if gelation takes place in an open vessel (i.e., ambient pressure) (Mehling et al., 2009), likely due to the extensive water evaporation occurring during thermal gelation. The starch gelation

Table 2

Physicochemical characterization of starch aerogel microspheres produced from water-in-oil emulsions at different oil:water volume ratios and gelation temperatures.

Gelation temperature, K	Oil-to-water phase ratio (v/v)							
	1:1				3:1			
	A_{BET}, m^2 g^{-1}	V_p, cm^3 g^{-1}	D_m, μm	$D(3,2)$, μm	A_{BET}, m^2 g^{-1}	V_p, cm^3 g^{-1}	D_m, μm	$D(3,2)$, μm
393	91 ± 5	0.34 ± 0.02	1159 ± 3	1030 ± 2	112 ± 6	0.36 ± 0.02	1226 ± 3	1081 ± 3
413	48 ± 2	0.12 ± 0.01	434 ± 1	129 ± 1	34 ± 2	0.13 ± 0.01	365 ± 3	75 ± 1

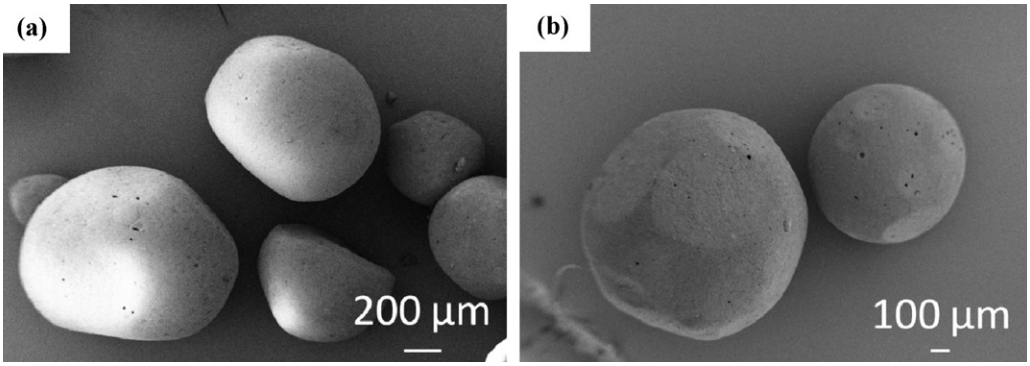

Fig. 6. SEM micrographs of starch microspheres obtained at the gelation temperature of 393 K and with different oil-to-starch aqueous solution ratios: (a) 1:1, 2:1 (Fig. 3a) and (b) 3:1 (v/v).

at 393 K and at a pressure above the water vapor pressure at this temperature is proposed to avoid the removal of structural water from the gel and thus getting superior textural properties. This approach resembles the liquid hot water (LHW) treatment used for the fractionation of lignocellulosic biomass as a pretreatment for bioethanol production in biorefineries (Zetzl, Gairola, Kirsch, Perez-Cantu, & Smirnova, 2011).

Water removal can also take place during solvent exchange and also during supercritical drying. Use of ethanol for the solvent exchange removes the water contained in the gel pores but is thought to play a secondary role in the dehydration of the structural water in the material backbone, since it has been already reported as a suitable solvent to preserve the starch gel structure (Budarin et al., 2006; Glenn et al., 2010). The removal of structural water by supercritical drying is not expected to be very intensive because of the low solubility of water in supercritical CO_2 (Sabirzyanov, Il'in, Akhunov, & Gumerov, 2002) and was mitigated using short drying times.

3.2.2. *Effect of oil-to-starch aqueous solution ratio*

The effect of the oil-to-starch aqueous solution ratio in the resulting morphology of the gel particles and the textural properties of the resulting starch aerogels was studied. The increase in the oil-to-starch aqueous solution ratio (oil:aqueous solution ratio = 1:1, 2:1, 3:1 (v/v)) during the emulsion gelation process gave rise to rounder particles (Fig. 3a and 6) with narrower particle size distribution. However, no significant effect of the oil-to-starch aqueous solution ratio on the mean particle size was observed (Tables 1 and 2). The increase in oil content led to a subsequent reduction of the volume of the dispersed phase and a decrease in the frequency of collisions and particle coalescence during agitation, resulting in the formation of less aggregated spherical microdroplets (Li et al., 2009). Moreover, there is a significant difference in viscosity between the oil phase (0.01–0.02 Pa s for canola oil) and the gelified starch (15–30 Pa s for native corn starch (Glenn et al., 2008; Mironescu & Schierle, 2004)). The increase of the oil-to-starch aqueous solution ratio thus decreased the viscosity of the emulsion and favors the homogeneization of the emulsion (Figs. 3a and 6). An increase in the gelation temperature (393 vs. 413 K) led to a dramatic decrease in the particle size of the gel particles for all the oil-to-starch aqueous solution ratios studied (Table 2). Finally, no influence of the oil-to-starch aqueous solution ratio during starch gel processing on the aerogel textural properties (A_{BET}, V_p, d_p in Table 2) were observed.

3.2.3. *Effect of surfactant*

The presence of the surfactant in the emulsion played a major role in the control of particle size of the porous starch microspheres. Glyceryl monostearate (GMS), a lipophilic surfactant (HLB = 4) commonly used in pharmaceutical and cosmetical products (García-González, Sampaio da Sousa, et al., 2009; García-González, Vega-González, López-Periago, Subra-Paternault, & Domingo, 2009; Sampaio de Sousa, Simplício, de Sousa, & Duarte, 2007), was added to the water-in-oil emulsion. The addition of GMS improves the stability of the emulsion until completion of gelation. The surfactant also contributes to the reduction in the droplet size of the dispersed phase by reduction of the surface tension between the aqueous and oil phases through its adsorption in the liquid–liquid interface. As expected, the use of a surfactant concentration of 3% (w/w) decreased the mean diameter of the gel microspheres (Fig. 4c and Table 1) regardless of the gelation temperature and the oil-to-starch aqueous solution ratio used (Table 3). The use of the surfactant did not have any significant influence on the textural properties of the resulting starch aerogel (Table 1). Finally, an increase in the GMS concentration did not lead to a further reduction of the particle size (Fig. 7).

Table 3

Mean particle size (in μm) of starch gel microspheres obtained by the emulsion-gelation mechanism using 3% of glyceryl monostearate as surfactant.

Gelation temperature, K	Oil–to-water phase ratio (v/v)		
	1:1	2:1	3:1
393	286 ± 2	519 ± 3	462 ± 4
413	–	215 ± 2	–

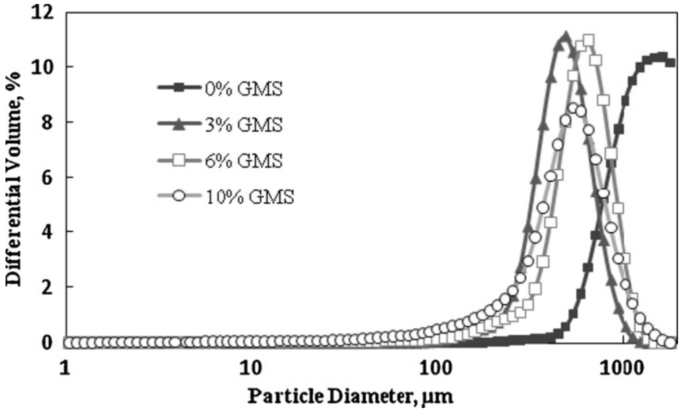

Fig. 7. Particle size distribution of starch gel particles at different surfactant concentrations from water-in-oil emulsions (2:1 oil:starch ratio (v/v)) processed at the gelation temperature of 393 K.

3.3. Drug loading capacity

The dissolution rate of chemicals can be improved by means of a decrease in their particle size, an increase in their surface area and/or a decrease in crystallinity (Overhoff, Johnston, & Williams, 2006). These three factors can be improved by adsorbing the chemical into high-surface area matrices as it is the case of aerogel particles. Moreover, the textural properties of aerogels (i.e., density, pore size, surface area) not only influence the drug adsorption behavior in the aerogel matrix, but also the maximum drug loading capacity of the aerogel (Smirnova et al., 2004, 2005).

The loading capacity of starch aerogel microspheres was evaluated by supercritical adsorption of ketoprofen. The loading of chemicals in the aerogel from a supercritical phase provides good mass transfer properties (diffusibility) and good solvation power (solubility of chemicals) while preserving the physical stability of the nanoporous material (Smirnova, Mamic, & Arlt, 2003; Smirnova et al., 2004, 2005). Ketoprofen was chosen as the model chemical to be loaded due to its high solubility in $scCO_2$ and that it is a hydrophobic non-steroidal anti-inflammatory drug commonly used for pharmaceutical and tissue engineering applications (García-González et al., 2010; García-González, Sampaio da Sousa, et al., 2009; García-González, Vega-González, et al., 2009; Macnaughton et al., 1996). The amount of ketoprofen loaded in the polysaccharide based matrix (starch aerogel processed at gelation temperature of 393 K, with 2:1 oil-to-starch aqueous solution ratio and no surfactant addition) was of 15.8 wt.% with a specific loading of 1.1×10^{-3} g m^{-2}. These values represent higher specific loadings than those reported in the literature for ketoprofen with silica aerogel as matrix ($2.8–3.8 \times 10^{-4}$ g m^{-2} (Smirnova et al., 2004)). Moreover, the original nanoporous structure of the starch aerogel was maintained after the ketoprofen was incorporated (Fig. 8). Textural results obtained with this supercritical fluid-assisted loading method contrast with the usual collapse or compression of porous structures due to capillary forces observed when similar amounts of chemicals are loaded from a liquid phase (i.e., dipping in a solution containing the chemical to be adsorbed) (Buisson, Hernandez, Pierre, & Pierre, 2001; Glenn et al., 2010). Finally, the absence of ketoprofen crystals on the surface of the starch aerogel particle (Fig. 8) seems to indicate the loading of the active compound in the amorphous form.

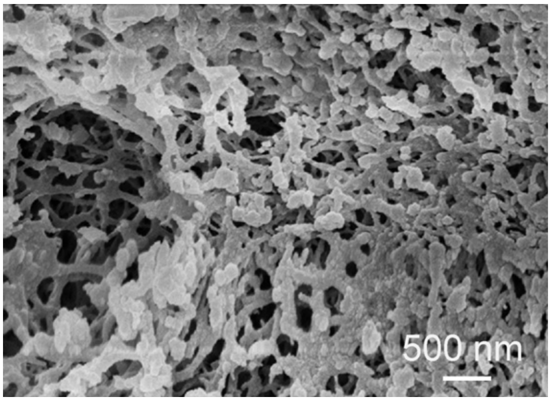

Fig. 8. SEM micrograph of the textural appearance of the nanoporous structure of a starch aerogel particle loaded with ketoprofen by supercritical fluid-assisted impregnation.

4. Conclusions

A novel one-pot method for the preparation of starch gel microspheres by thermal gelation of starch solution-in-oil emulsions with no use of chemical crosslinkers was developed. The microdroplets of the dispersed aqueous phase in the emulsion-gelation method allowed the templating of the starch gels in the form of microspheres. Supercritical drying of the starch gels proves to be an excellent method to preserve the inherent porous structure of the wet gels in a dry form. Gelation temperature was the main processing parameter influencing the textural properties of the end aerogel material (A_{BET} = 112 m^2 g^{-1}, V_p = 0.37 cm^3 g^{-1} at 393 K; A_{BET} = 40 m^2 g^{-1}, V_p = 0.18 cm^3 g^{-1} at 413 K). Higher oil-to-starch aqueous solution ratios in the emulsion and the use of surfactants enhanced the sphericity of the particles and reduced the particle size by 250%, respectively. Starch aerogel particles showed high loading capacities (16 wt.%) of a model chemical (ketoprofen) in the amorphous form. The release profiles of chemicals loaded in the starch aerogel matrices are currently under investigation. The preparation of starch aerogel microspheres herein presented (use of biocompatible and biodegradable precursors and avoidance of toxic solvents and chemical crosslinkers) allows the application of these carriers in life science applications without further post-processing (e.g., purification) treatments.

Acknowledgments

C.A. García-González acknowledges the Spanish Ministry of Education for the financial support through a postdoctoral fellowship in the frame of the National Program for Staff Mobility from the R&D&i National Plan 2008–2011. Authors are grateful to Frank Rimoschat (research group of Prof. Heinrich, Institute of Solids Process Engineering and Particle Technology, Hamburg University of Technology) for his technical support with the measurements of particle size distribution by laser diffraction. The support of Dr. Robin White with the TGA and CHN analyses is greatly acknowledged. The authors want to acknowledge Roquette Frères (Dr. O. Häusler) for the supply of the corn starch and to Lambent Technologies for the supply of the glyceryl monostearate. Chemische Fabrik Kreussler & Co. GmbH is acknowledged for the supply of the pharmaceutical grade ketoprofen.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2012.02.023.

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