Polysaccharide-based aerogels: Promising biodegradable matrices for life sciences applications

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Natural polymers and derivatives are attractive feedstocks for life science applications (e.g. pharmaceutics, tissue engineering, cosmetics, food, biotechnology, agriculture) because of their biodegradability, biocompatibility, stability, availability and renewability. Namely, the broad portfolio of biocompatible polysaccharides with different functional groups (e.g. carboxylic, sulfonic, hydroxyl and amino groups) and behaviours (anionic, cationic, non-ionic) is a promising starting point for the development of tailor-made materials with controlled release properties. Moreover, aerogels are being studied for life sciences applications because of their light-weight, outstanding surface area, and open porosity, susceptible to be loaded with active compounds. In this work, the ability of polysaccharide to form stable hydrogels was exploited to get polysaccharide-based aerogels from different precursors (starch, alginate, pectin) by the supercritical carbon dioxide ($scCO_2$)-assisted drying process of gels. The development of different production strategies (moulding, dropping and emulsion technologies) to obtain aerogels of different morphologies and sizes (cylindrical monoliths, spherical milimeter-sized beads and microspheres, respectively) was carried out.

1. INTRODUCTION

Natural polymer-based (bio-based) materials have the great advantage of being a renewable and suitable feedstock for environmentally sustainable processes. These properties make natural polymers adequate for a wide range of life sciences applications, ranging from agricultural (e.g. pesticides) to pharmaceutical (e.g. drug delivery systems) including food and biotechnology industries [1]. Among them, the use of natural polysaccharides and/or their derivatives is especially attractive because of their availability, renewability and low toxicity. Moreover, polysaccharides are usually biodegradable, biocompatible and biologically stable. These properties coupled with their capacity for chemical modification confers them ideal properties for their use in drug release systems with different routes of delivery, target organs and/or drug release profles.

Polysaccharides are used as solid matrices to incorporate drugs in different forms, such as monoliths, beads, micro- or nanoparticles, depending on the pharmaceutical formulation. For these purposes, the drug loading capacity is largely influenced by the chemical structure of the matrix, the porosity and the surface area of the polysaccharide-based matrix. In this sense, aerogels present highly porous, light-weight structures with large surface areas that enhance the maximum drug loading capacity of the aerogel as well as influence the drug adsorption behavior in the aerogel matrix [2]. The ability of polysaccharides to undergo gelation by different external stimulii (e.g. temperature, counterions, covalent cross-linkers) using sol-gel technology allows the production of aerogels after $scCO_2$-assisted drying of the wet gels [3].

The choice of the supercritical drying of gels attends to the suitability of this technology to preserve the original nanoporous structure of the wet polysaccharide gel in a dry form (i.e, aerogel).

In this work, polysaccharide-based aerogels were prepared in different morphologies (cylinders, beads and microspheres) using different production strategies (moulding, dropping and emulsion technologies). Case studies for each strategy were selected accordingly, to test the versatility of the processing methods for polysaccharides with different gelation mechanisms (starch, alginate and pectin gels). Finally, the obtained aerogels were assessed for their use as carriers of a model compound (ketoprofen) by $scCO_2$-assisted impregnation.

2. MATERIALS AND METHODS

2.1. REAGENTS

Na-alginate was purchased from Sigma. Native corn starch was provided by Roquette. High methoxyl (HM) and low methoxyl (LM) pectin were provided by Pektowin. Ethanol 99.8% and glacial acetic acid were purchased from Merck GmbH, Germany. $CaCO_3$ was kindly provided by Magnesia GmbH Germany. Carbon dioxide with a purity of >99.9% was supplied by AGA Gas GmbH (Hamburg, Germany).

2.2. PROCESSING

The processing steps needed for the production of polysaccharide-based aerogels are summarized in Figure 1 and described in the following subsections. Briefly, aerogel processing starts with the formation of a gel from an aqueous solution, i.e., a hydrogel (Section 2.2.1). The next step is the replacement of the water filling the pores of the gel structure by ethanol to lead to an alcogel (Section 2.2.2). Finally, ethanol is extracted from the gel by $scCO_2$-assisted drying and the aerogel end material is obtained.

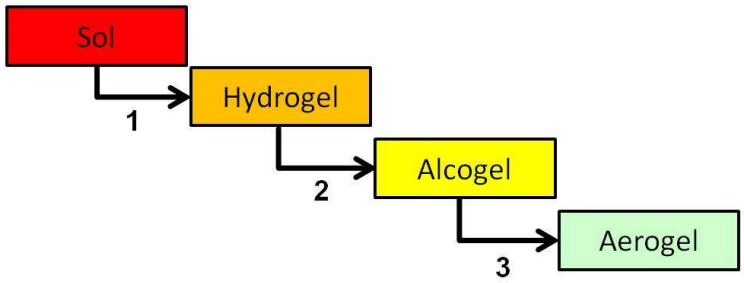

Fig. 1: Schematic depiction of the supercritical drying process for the preparation of aerogels: *1*: Gelation; *2*: Solvent exchange from water to ethanol; *3*: Supercritical CO_2-assisted drying of gels.

2.2.1. Gel preparation

The gel formation from a solution (sol) is induced by a cross-linking promoter that can be of chemical (e.g. cross-linker compound) or physical (e.g. pH, temperature) nature. The nature of the cross-linker and the mode of addition to the solution to induce gelation determine the gelation kinetics and the processing strategies available to obtain gels of different

morphologies and sizes. The three processing strategies used in this work are described in the following subsections.

2.2.1.1 Cylindrical gels preparation by moulding

Starch and HM-pectin form thermotropic gels, i.e., they have temperature-induced gelation. For the preparation of the thermotropic gel monoliths [4], a dispersion of the polysaccharide precursor in water was prepared under agitation (500 rpm). Polysaccharide dispersion was then heated up (120°C and 70°C for starch and pectin, respectively) in an autoclave equipped with magnetic stirring (300 - 500 rpm). Once the desired temperature was reached, the operating conditions were kept constant for 20-35 min. Then, the viscous polysaccharide solution was poured into syringes (1.20 cm diameter) acting as moulds and sealed. The starch samples were placed in the fridge for retrogradation at 277 K for 48 hours and pectin gels were left under ambient conditions.

2.2.1.2 Bead gels preparation by dropping

The ionotropic gelation of LM-pectin was carried out by dropping a solution containing the polysaccharide aerogel precursor into a solution containing the gelling promoter agent (Ca^{2+} cations) [5]. Briefly, an homogenous and bubble-free aqueous stock solution of LM-pectin (4% w/v) was dropped through a disposable syringe (0.6 mm inner diameter of nozzle) at an average rate of 1 mL/min, into 50 ml solution of cross-linking agent (0.15 M of $CaCl_2$) with pH 1.6 (adjusted by 1 N HCl) stirred at 150 rpm. The formed bead gels were allowed to age in the cross-linking solution for 24 h and then separated by filtration and washed three times with deionized water.

2.2.1.3 Microspherical gels preparation by emulsion polymerization technique

The emulsion-gelation method was used to obtain the alginate gel microspheres using $CaCO_3$ as source of Ca^{2+} cations, the gelation promoter [6]. Briefly, the alginate stock solution was mixed vigorously with 5 wt.% $CaCO_3$ ($CaCO_3$/alginate solution) to attain a Ca^{2+}-to-Na-alginate weight ratio of 7.3. Then, the dispersion containing the alginate (dispersed phase) is added to paraffin oil (continuous phase) containing Span 80 (1 vol%) as surfactant to reach a water-in-oil emulsion (1:2 w/o phase mass ratio (w/w)). The emulsion was then stirred for 15 min. Then, a mixture of paraffin oil and glacial acetic acid (acid-to-Ca^{2+} molar ratio of 3.5) was added to the system to induce cross-linking. Alginate solution droplets gelled due to the release of the Ca^{2+} cations from the salt with the reduction of the dispersion pH caused by the addition of glacial acetic acid. Finally, the microspherical particles were harvested using filter paper.

2.2.2 Solvent exchange

Solvent exchange for starch and pectin hydrogels was carried out by soaking the gel directly in ethanol (one-step) transferring to fresh ethanol (second solvent exchange) after 24 h. The solvent exchange of alginate gels was performed by following a sequential soaking (multi-step) in different water-to-ethanol mixtures with increasing content in the new solvent (30:70, 50:50, 70:30, 100:0 (x2) ethanol-to-water volume ratio) after a certain time (3 h of exchange frequency) in the previous soaking step.

2.2.3. Supercritical drying

The resulting alcogels in the form of cylinders, beads or microspheres were dried by extraction of the solvent with a continuous flow of $scCO_2$ using the equipment sketched in the process flow diagram of Fig. 2. In a typical experiment, the alcogels immersed in ethanol are placed into a 250 ml-autoclave (E1 in Fig. 2). The autoclave was heated to 40°C and CO_2 was fed by a compressor (P1) to the autoclave until the desired working pressure (11.0-12.0 MPa) was reached. Then, the outlet valve (V4) of the autoclave was opened and the outlet flow rate was regulated by the micrometering valve V5 to 2-4 Nl/min. The alcogels were dried under these operating conditions for 4 h. Finally, the pressure was released until atmospheric pressure was reached.

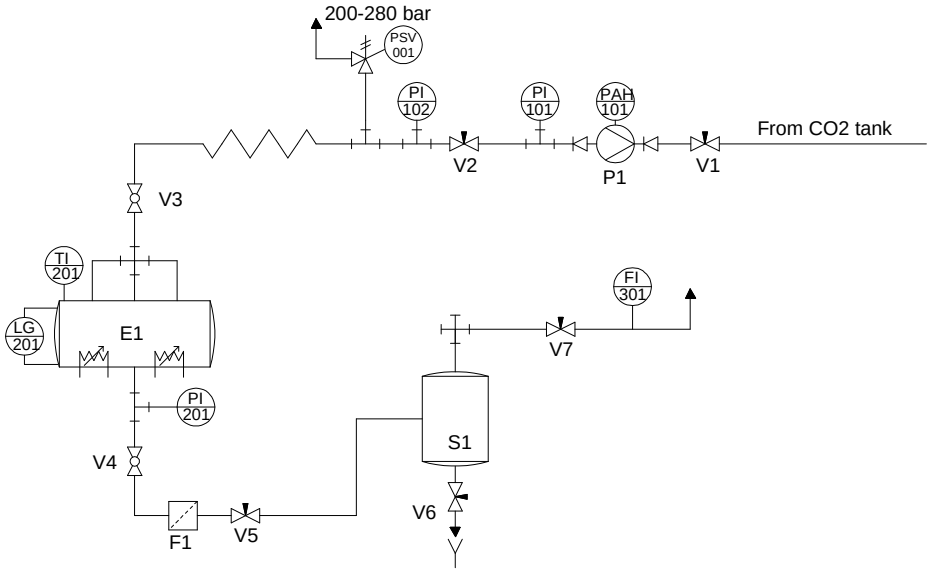

Fig. 2. Process flow diagram of the equipment used for the supercritical drying of polysaccharide-based aerogels with $scCO_2$

2.3. CHARACTERIZATION

Aerogels were characterized using different methods to evaluate the process and the effect of processin parameters in the end material properties. Textural characterization of the polysaccharide aerogels was carried out by low-temperature N_2 adsorption-desorption analysis (Nova 3000e). Particle size distribution of the wet microspheres dispersed in ethanol was measured using laser diffraction spectrometer (Beckman Coulter LS1332). Aerogel shape and appearance were analyzed using scanning electron microscopy (SEM) (Leo Zeiss 1530).

3. RESULTS

3.1. STARCH AND HM-PECTIN AEROGEL MONOLITHS

The preparation of aerogels involves the elimination of the liquid solvent from the gel, whilst avoiding the collapse of the already existing nanoporous structure. Traditional drying procedures, e.g. air drying, are not able to preserve the gel structure (*1* in Fig. 3) leading to pore collapse and massive shrinkage (xerogels) (*3* in Fig. 3). This drying method form liquid–vapor menisci in the pores of the gel which recedes during emptying of the pores of the wet gels. Upon solvent removal, the surface tension of the liquid contained in the gel nanopores

will create a capillary pressure gradient in the pore walls able to collapse the pores. Alternatively, supercritical drying process is a drying method able to preserve the high open porosity and superior textural properties of the wet gel in a dry form leading to aerogels (*2* in Fig. 3). In the supercritical drying process, there are no liquid/gas interfaces in the pores during drying, thus, avoiding the strong capillary forces associated with air drying.

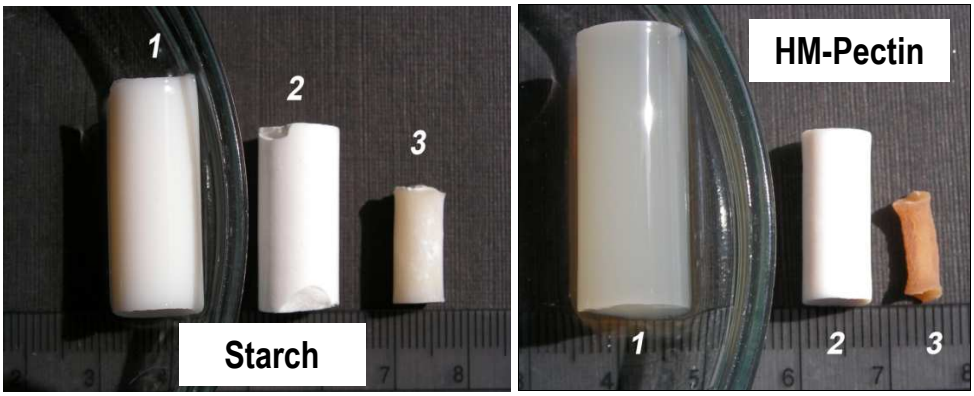

Fig. 3: Effect of gel drying method: Gel monoliths (*1*, wet gel) of corn starch (left) and high-methoxyl pectin (right) of the same dimensions dried under supercritical drying (*2*, aerogel) and under air drying (*3*, xerogel)

The effect of the concentration of the polysaccharide in the *sol* (precursor concentration) on the resulting end aerogel properties was studied for two thermotropic gels: corn starch and HM-pectin. Other processing parameters (e.g. temperature) may also strongly influenced the aerogel end properties [3]. The higher mechanical stability of the aerogels processed from hydrogels with higher polysaccharide content highly influenced the volume reduction of the cylinders after the overall process (i.e., total shrinkage: shrinkage after solvent exchange plus supercritical drying) (Fig. 4). The total shrinkage after corn starch aerogel processing fell down from 49 to 25% as the starch content in the original *sol* increased from 7 to 15% (Fig. 4a). Similarly, the total shrinkage of cylindrical HM-pectin aerogels decreased from 64 to 52% with the increase in pectin content of the stock solution from 3 to 5% (Fig. 4b).

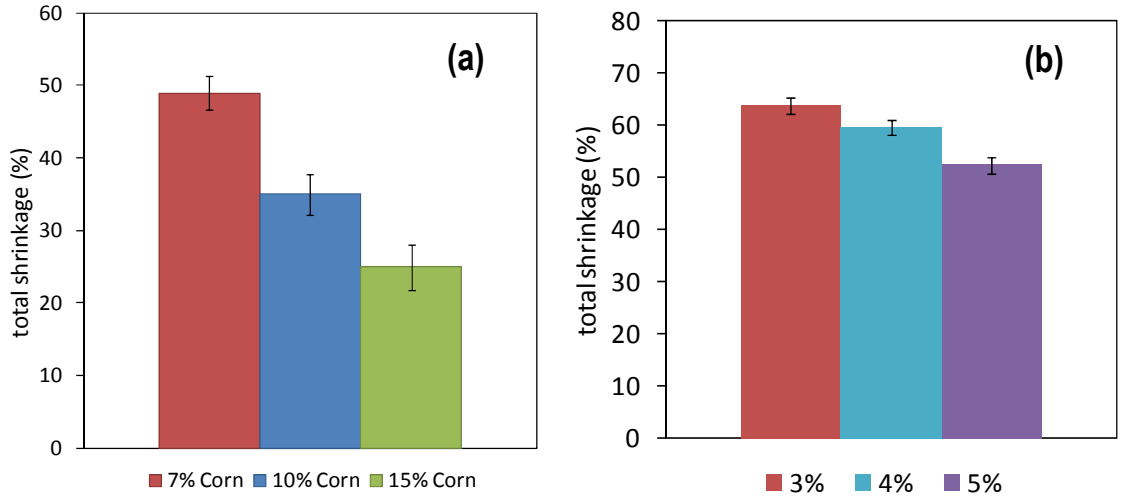

Fig. 4: Total shrinkage of aerogel monoliths obtained from polysaccharide solutions of (a) corn starch (7, 10 and 15 wt %) and (b) HM-pectin (3, 4 and 5%)

The more extensive volume shrinkage obtained for starch aerogels with lower precursor concentration partially compensated the influence in the aerogel density of the initial precursor concentration. Consequently, the density of the starch aerogel cylinders increased with the precursor concentration, but is not linearly as it would be initially expected (Fig. 5a). Porosity of starch aerogels correlated well with the aerogel density and gave the highest values (90%) for the aerogel with the lowest density (0.154 g/cm^3 for 7% corn starch) (Fig. 5b). The specific surface area of the resulting starch aerogels were in the range of 240-260 m^2/g. Similar trends with the precursor concentration were observed for HM-pectin aerogels (not showed). The resulting HM-pectin aerogels were porous (96-97%) and light-weight (0.04-0.06 g/cm^3) cylinders with specific surface areas in the range of 280-300 m^2/g depending on the initial pectin content in the *sol*.

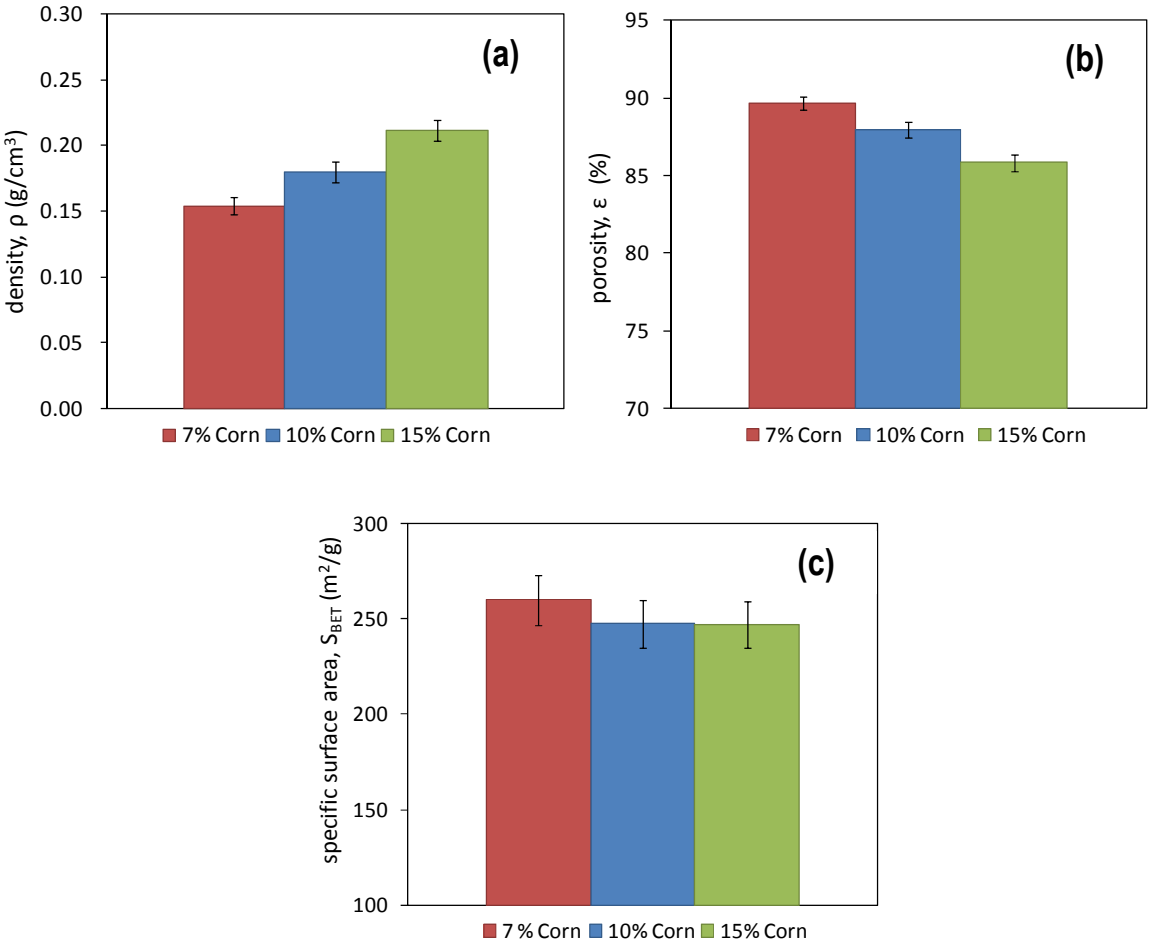

Fig. 5: Effect of the corn starch concentration on the end aerogel properties: (a) density, (b) porosity and (c) specific surface area

3.2. LM-PECTIN AEROGEL BEADS

LM-pectin aerogel beads were obtained by dropping the aerogel precursor into a solution containing the gelling promoter agent (Ca^{2+} cations) and subsequent solvent exchange and supercritical drying. The aerogel beads showed differences in shape and uniformity depending on the processing approach. The injection mode of the LM-pectin solutions strongly influences the roundness of the end aerogel beads, due to different contributions on the droplet deformation of impact and drag forces exerted when the droplet hits and enters the

gelling bath.Results obtained showed that, for the same falling distance, LM-pectin aerogel bead deformation is more significant if the syringe used for injection is placed horizontally (Fig. 6a) instead of vertical (Fig. 6b). Moreover, the roundness of the resulting gel beads is enhanced by decreasing the falling distance (Fig. 6c). Nevertheless, the injection mode influenced the end aerogel morphology, but not significantly the end textural properties of the LM-pectin aerogel (not showed).

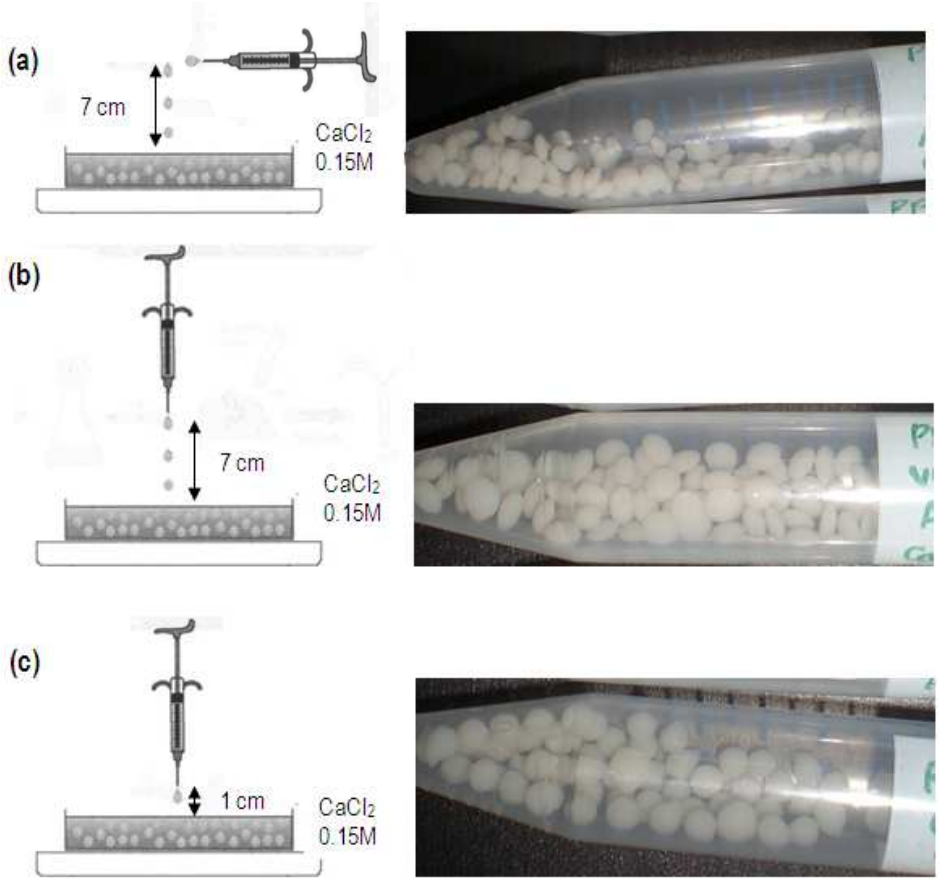

Fig. 6: Effect of the injection mode (left) on the resulting appearance of LM-pectin aerogels (right): syringe containing the polysaccharide solution placed (a) horizontally with respect to the gelling promoter solution, or vertical at the falling distances of (b) 7 cm and (c) 1 cm

3.3. ALGINATE AEROGEL MICROSPHERES

Aerogel microspheres were obtained by coupling the emulsion techniques to the conventional aerogel technology. Using this approach, spherical aerogel particles (diameter of 20–600 µm) were obtained [6] (Fig. 7a). Properties of the microspheres (particle size, density, surface area) can be tailored by controlling the processing conditions and chemical formulation. The size distribution of the gel particles are mainly influenced by agitation (Fig. 7b), surfactant concentration, matrix precursor concentration and aqueous solution-to-oil volume ratios. The textural properties (surface area, pore size) of the resulting aerogel microspheres were not significantly influenced by the processing conditions (stirring rate, aqueous solution-to-oil ratio, surfactant concentration), but mainly by the composition of the sol itself (precursor concentration, crosslinker) [6]. Moreover, the surface area and the pore size distribution of the aerogel microparticles show similar values (S_{BET}=590 m^2/g, $V_{p\text{-}BJH}$=4.10 cm^3/g) to those obtained with the corresponding monolithic aerogels (not showed).

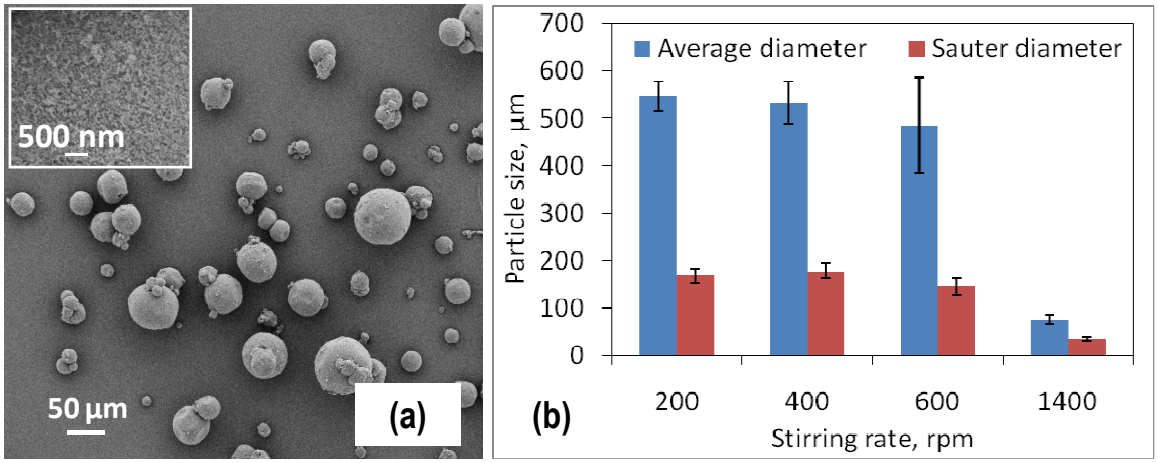

Fig. 7: Alginate aerogel microspheres: (a) SEM picture (top-left: magnification) and (b) influence of stirring rate during emulsion in the alginate gel particle size.

3.4. DRUG LOADING CAPACITY

The drug loading capacity of polysaccharides was tested by supercritical impregnation of ketoprofen 50°C and 20.0 MPa during 6 h and subsequent photometrical quantification of the drug content by UV/Vis. Obtained results showed drug loadings in the range of 6 to 9 wt.% depending on the polysaccharide source and the processing parameters used for the aerogel formation. The determination of the maximum drug loading capacity is currently under investigation using longer impregnation times.

4. CONCLUSION

Aerogels from polysaccharides exhibiting different gelation mechanisms (thermotropic –starch–, ionotropic –alginate–, and thermo+ionotropic –pectin– gels) were prepared. Supercritical drying process is a suitable drying technique to preserve the high open porosity and superior textural properties of the wet polysaccharide gel in a dry form. The size and morphology of polysaccharide aerogels was customized by means of shaping of the wet gel by molding, dropping or templating in the dispersed phase of an emulsion. The overall result for all morphologies is an aerogel product with tunable properties (density, surface area, particle size) achieved by means of the control of the processing conditions and chemical formulation. The high specific surface areas (100-700 m^2/g) obtained for the aerogels gave loadings of drug (ketoprofen) of up to 9 wt.% by supercritical impregnation.

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