



Green Processing of Nanoporous Biodegradable Carriers of Bioactive Agents for Pharmaceutical and Biomedical Applications

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Abstract: Pharmaceutical and biomedical industries demand simple, safe and reproducible processing methods thus urging the development of novel straightforward manufacturing approaches. The product manufacturing by the green processing of admixtures and end-product would avoid long and costly purification (downstream) steps. In this work, the supercritical fluid technology is used for the green processing of nanoporous carriers (aerogels) for bioactive agents [1,2]. Aerogels in the form of one micron-sized particles were processed and loaded with a model bioactive compound (ketoprofen). Results show that the carrier has excellent textural properties (specific surface area of 205 m²/g) and a good loading capacity (8 wt.%) of the bioactive compound in the amorphous form. Release profile tests show the capacity of the carrier to modulate the drug release to the medium (pH 1.2 and 7.4). The resulting material can be potentially incorporated in the formulation of several pharmaceutical and biomedical products.

Keywords: supercritical fluid technology; green processing; aerogel; nanoporous material; bioactive agent carrier

1. Introduction

Nanotechnology research is facing the challenge of reaching mass markets in the near future by providing innovative and sustainable solutions to the demands of the society. Indeed,

smart, reproducible and environmentally friendly solutions for nanotechnology are being sought in the life sciences field to be aligned with current and forthcoming environmental regulations. In

this context, pharmaceutical and biomedical industries are focusing part of their research and innovation on the efficient use of raw materials and on the development of novel green processes with low environmental impact.

Supercritical fluid (SCF) technology emerges as a green processing method of nanomaterials. The possibility of modulation of the physicochemical properties of SCF (e.g., density, viscosity, diffusivity, dielectric constant) allows the processing with a fine control of the nanomaterial end properties. Moreover, solvent-free nanomaterials are obtained using this technology without need of downstream processes. Supercritical CO₂ is the most common choice of SCF attending to its low cost, health and safety properties (innocuous, non-flammable, GRAS substance) and the mild operating conditions needed to reach its critical point (31.1°C, 73.8 bar). Finally, CO₂ used in supercritical processes can be potentially reused in a closed loop without contribution to greenhouse gas emission.

Aerogels are a special class of nanostructured materials obtained using SCF technology [1]. Aerogels have high porosity (>90%) in the mesoporous range and with outstanding textural properties (specific surface areas higher than

2. Results and Discussion

Starch aerogel particles were obtained as a fine white powder with a particle size distribution in the 200 nm to 1.5 µm range and a mean particle size of 1.110±0.149 µm. Aerogel powder was free-flowing with an angle of repose of 34° and a compressibility of 35%.

SEM pictures highlight the presence of spherical microparticles (Fig. 1,top). Primary particles were loosely joined together forming agglomerates likely due to the presence of emulsifier (PGPR) remnants.

150m²/g). These nanomaterials are obtained by the supercritical extraction of the solvent contained in gels [2], the unique technique able to preserve the porous gel network in the dry form. Among the aerogel types, polysaccharide aerogels are especially attractive for life sciences purposes since it combines the above-mentioned intrinsic properties of aerogels with the availability, renewability, low toxicity and usual biodegradability and biocompatibility of polysaccharides [3, 4]. Finally, the preparation of aerogels in the form of fine powder would facilitate the incorporation of the material as an admixture in pharmaceutical and biomedical formulations [5, 6].

In this work, starch aerogels in the form of one-micron particles are processed by a combination of emulsion-gelation and supercritical drying techniques. Morphological and textural properties of aerogels are evaluated. Finally, aerogel particles are loaded with an anti-inflammatory drug (ketoprofen) by supercritical impregnation and the drug release from the aerogel is evaluated using simulated gastric (pH 1.2) and blood (pH 7.4) conditions to assess its behavior for different administration routes.

The nanoporous structure of the aerogel particles processed by supercritical drying can be observed at the highest magnification (Fig. 1,bottom). Excellent textural properties of the aerogel powder were obtained with specific surface areas of 205 m²/g (BET-method), specific pore volume of 0.98 cm³/g and mean pore size of 17 nm (BJH-method). In contrast, specific surface areas below 5 m²/g was obtained for the same gel particles dried under ambient

conditions, highlighting the high performance of the supercritical drying method.

The poor solubility of ketoprofen in water and its good solubility in supercritical CO₂ suggested the use of supercritical impregnation of aerogels

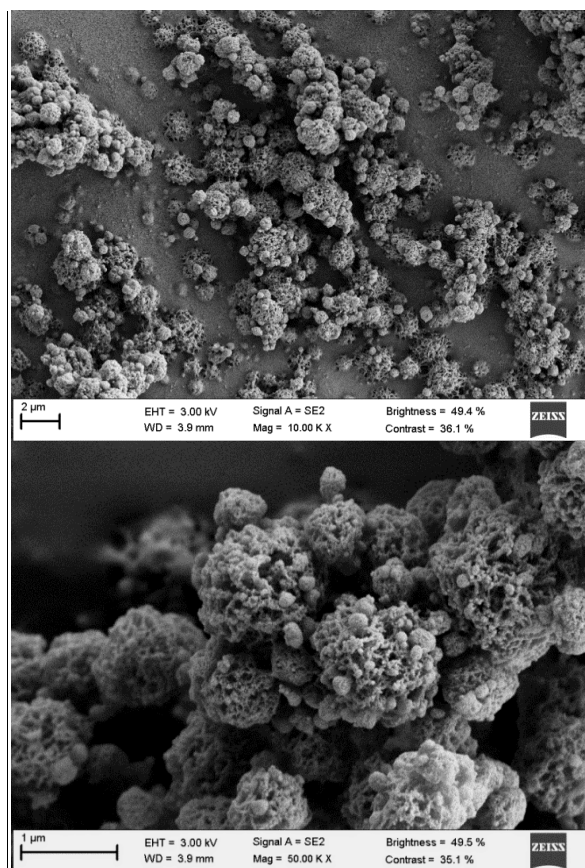


Figure 1. SEM images of starch aerogel microspheres (top). Higher magnifications show the nanoporous structure of the particles (bottom).

as the best choice for the drug loading. The use of this solvent-free method led to a high ketoprofen loading (*ca.* 8 wt.%) in the aerogel matrix. Supercritical impregnation of drugs in aerogels usually leads to the adsorption of the drug in the amorphous form [6]. The ketoprofen amorphization and its loading in the aerogel matrix significantly increased the drug release rate with respect to the raw ketoprofen in both simulated physiological media (pH 7.4 and pH 1.2) (Fig. 2). Ketoprofen loaded in the aerogels

was released faster at pH 7.4 than at pH 1.2 as also observed with the raw crystalline drug. This behaviour was related to the pK_a value of ketoprofen (4.4) in-between the two pH media studied thus leading to different ionic forms of the drug in the two simulated physiological conditions studied. After an initial burst during the first 15 min, the release profile of ketoprofen from the aerogel was almost linear at pH 1.2 during the release period studied (Fig. 2a). At pH 7.4, a two-stage release profile is observed for ketoprofen loaded in the aerogels (Fig. 2b): a fast release profile of *ca.* 60% of the ketoprofen payload during the first 2 h is followed by a slower release in the following hours.

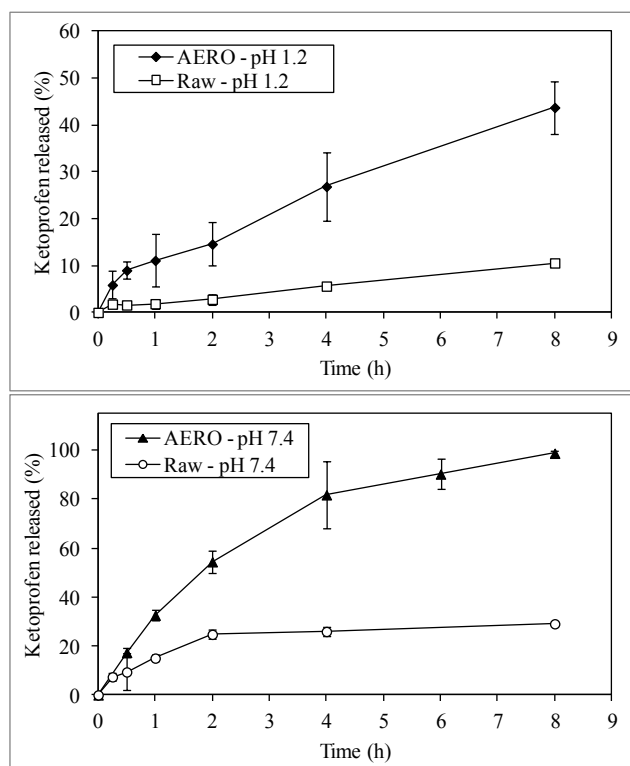


Figure 2. *In vitro* release profiles of ketoprofen from starch aerogel microspheres (dark markers) at two different pH media: pH 1.2 (top) and pH 7.4 (bottom). Release profiles of the raw ketoprofen (blank markers) are also plotted for the sake of comparison

3. Materials and Methods

3.1. Reagents and chemicals

Native corn starch (Starch Amylo N-460; 52.6% amylose content) was from Roquette (France). Ketoprofen (Raw, 99.7% purity) was provided by Acofarma (Spain). Ethanol (99.8% purity) was from Omnilab (Germany). Polyglycerol polyricinoleate (PGPR) was from Palsgaard (Denmark). Paraffin oil was provided by Panreac (Spain). CO₂ (99.8%) was obtained from Praxair (USA).

3.2. Methods

3.2.1. Aerogel preparation

Starch gel microspheres were prepared by the method of emulsification-gelation [4]. A water-in-oil emulsion (W/O) was prepared from a mixture of paraffin oil (continuous phase)/aqueous starch solution 15% (w/w) (dispersed phase) in the 3:1 weight ratio and 3% (w/w) of emulsifier. The mixture obtained was autoclaved (Trade Raypa Steam Sterilizer, USA) at 121°C and 1.1 bar for 20 min. After sterilization and partial cooling (95 °C), the mixture was homogenized using an ultrasound probe (Branson Digital Sonifier; Mexico) and kept for 48 h at 4 °C for starch retrogradation. The resulting starch hydrogel dispersion in paraffin was centrifuged to separate the hydrogel from the oil phase. Starch hydrogel microspheres were transferred to a fresh solution of ethanol for solvent exchange, which was replaced daily for

two days until complete removal of water to get an alcogel.

Aerogel microspheres (AERO) were obtained by the scCO₂-assisted drying of the starch gels with a CO₂ flow of 5-7 g/min during 3.5 h in a 100-mL autoclave (TharSFC, USA). The autoclave was heated to 40°C and the pressure increased progressively until 120 bar.

Particle size of the aerogel particles were measured by the dynamic light scattering method (Malvern Zetasizer Nano ZS, UK). Textural properties were obtained by a low-temperature N₂ adsorption-desorption analysis (ASAP 2000 Micromeritics Inc., USA).

3.2.2. Aerogel impregnation and release studies

Aerogels were impregnated with ketoprofen by the supercritical CO₂-assisted impregnation process at 40°C and 150 bar for 11 h in the batch mode.

Release of ketoprofen was performed using dialysis membranes (Float-A-Lyzer, MWCO 8-10 KD, V 1ml; USA) in 100 mL of PBS solution (pH 7.4) and 0.1N HCl aqueous solution (pH 1.2) as release media. Release studies were carried out at 37°C and at the stirring rate of 100 rpm for 8 h using 10 mg of sample. 2 ml-aliquots are collected at selected times (0.5, 1, 2, 4 and 8 h) and refilled with fresh medium. Ketoprofen concentrations were spectrophotometrically determined by UV/Vis at the wavelength of $\lambda=258$ nm.

4. Conclusions

Green processing approaches have been used for the preparation of nanoporous starch (aerogel) microparticles. One-micron aerogel microspheres with excellent textural properties were obtained. Supercritical impregnation technique allowed the loading of the aerogels with drugs (8 wt.% for ketoprofen). Ketoprofen release results highlighted the significant effect of the drug incorporation into starch aerogels with respect to the raw crystalline drug in enhancing the ketoprofen dissolution properties at the two experimental pH conditions tested (1.2 and 7.4). The drug-loaded aerogels can be

potentially incorporated in the formulations of several pharmaceutical and biomedical products to improve the drug release profile.

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Conflicts of Interest

The authors declare no conflict of interest.

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