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Abstract: Polysaccharide-based aerogels in the form of microspheres were investigated as carriers of poorly water soluble drugs for oral administration. These bio-based carriers may combine the biocompatibility of polysaccharides and the enhanced drug loading capacity of dry aerogels. Aerogel microspheres from starch, pectin and alginate were loaded with ketoprofen (anti-inflammatory drug) and benzoic acid (used in the management of urea cycle disorders) via supercritical CO2-assisted adsorption. Amount of drug loaded depended on the aerogel matrix structure and composition and reached values up to 1.0×10-3 and 1.7×10-3 g/m2 for ketoprofen and benzoic acid in starch microspheres. After impregnation, drugs were in the amorphous state in the aerogel microspheres. Release behavior was evaluated in different pH media (pH 1.2 and 6.8). Controlled drug release from pectin and alginate aerogel microspheres fitted Gallagher-Corrigan release model (R2>0.99 in both cases), with different relative contribution of erosion and diffusion mechanisms depending on the matrix composition. Release from starch aerogel microspheres was driven by dissolution, fitting the first-order kinetics due to the rigid starch aerogel structure, and showed different release rate constant (k1) depending on the drug (0.075 and 0.160 min-1 for ketoprofen and benzoic acid, respectively). Overall, the results point out the possibilities of tuning drug loading and release by carefully choosing the polysaccharide used to prepare the aerogels.

HIGHLIGHTS

- Polysaccharide aerogel microspheres are investigated as carriers of drugs for oral administration
- Aerogels were loaded with ketoprofen and benzoic acid, poorly water soluble model drugs
- Starch, with the lowest specific surface area, was more prone to adsorb drug molecules
- Release of ketoprofen from alginate and pectin aerogel particles was sensitive to pH of the medium
- Results point out the possibilities of polysaccharide aerogels of tuning drug loading and release

Polysaccharide-based aerogel microspheres for oral drug delivery

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Abstract

Polysaccharide-based aerogels in the form of microspheres were investigated as carriers of poorly water soluble drugs for oral administration. These bio-based carriers may combine the biocompatibility of polysaccharides and the enhanced drug loading capacity of dry aerogels. Aerogel microspheres from starch, pectin and alginate were loaded with ketoprofen (anti-inflammatory drug) and benzoic acid (used in the management of urea cycle disorders) via supercritical CO_2-assisted adsorption. Amount of drug loaded depended on the aerogel matrix structure and composition and reached values up to 1.0×10^{-3} and 1.7×10^{-3} g/m^2 for ketoprofen and benzoic acid in starch microspheres. After impregnation, drugs were in the amorphous state in the aerogel microspheres. Release behavior was evaluated in different pH media (pH 1.2 and 6.8). Controlled drug release from pectin and alginate aerogel microspheres fitted Gallagher-Corrigan release model (R^2>0.99 in both cases), with different relative contribution of erosion and diffusion mechanisms depending on the matrix composition. Release from starch aerogel microspheres was driven by dissolution, fitting the first-order kinetics due to the rigid starch aerogel structure, and showed different release rate constant (k_1) depending on the drug (0.075 and 0.160 min^{-1} for ketoprofen and benzoic acid, respectively). Overall, the results point out the possibilities of tuning drug loading and release by carefully choosing the polysaccharide used to prepare the aerogels.

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1 Introduction

Bio-based materials may become key formulation ingredients for the engineering of delivery systems able to overcome the biopharmaceutical and stability limitations shown by a number of well-established drugs and new chemical entities (García-González, Alnaief & Smirnova, 2011; García-González, Argemí, Sousa, Duarte, Saurina & Domingo, 2010; Pose-Vilarnovo et al., 2004). Natural polysaccharides and/or their derivatives are especially attractive because of their availability, renewability, low toxicity, stability upon storage, good biological performance, and enzyme-controlled biodegradability (Baldwin & Kiick, 2010; Dumitriu, 2012; García-González, Alnaief & Smirnova, 2011; Veronovski, Knez & Novak, 2013). Technological advances in extraction and purification processes enable the cost-effective obtaining of natural polysaccharides in large-scale, rendering them interesting for life science applications, e.g., pharmacy, tissue engineering and environmental remediation (Alvarez-Lorenzo, Blanco-Fernandez, Puga & Concheiro, 2013; Baldwin & Kiick, 2010; Kayser, Müller, García-González, Smirnova, Leitner & Domínguez de María, 2012). Several polysaccharide-based drug delivery systems (e.g., Lupron Depot® and Nutropin Depot®) can already be found in the market (Kim & Pack, 2006).

Polysaccharide-based aerogels for drug delivery and biomedical systems were firstly proposed by Berg et al. (Berg, Droege, Fellmann, Klaveness & Rongved, 1995) and since then there has been an increasing interest on the research of these materials for pharmaceutical technology purposes (García-González, Alnaief & Smirnova, 2011; Ulker & Erkey, 2014). Aerogel technology provides high added-value lightweight materials with outstanding surface area and open porosity, suitable to be loaded with active substances. Organic aerogels prepared with US Food and Drug Administration (FDA)- and European Medicines Agency (EMA)-approved bio-based polysaccharides offer unique features as drug carriers by combining the intrinsic properties of the aerogel structure and those of the polysaccharides. Moreover, aerogel carriers may load high amounts of drugs, improve their stability, and control the crystalline form of the drug (García-González & Smirnova, 2013; Smirnova, Suttiruengwong & Arlt, 2004). Drug loading within starch aerogels has been reported to be in the range 1–4 × 10^{-3} g/m^2 for ibuprofen in starch aerogel monoliths (Mehling, Smirnova, Guenther & Neubert, 2009) or ketoprofen in starch aerogel particles (García-González & Smirnova, 2013), which

is larger than the values obtained for silica aerogels (3.8×10^{-4} g/m^2), an aerogel reference material. Using the supercritical fluid-assisted drug impregnation, drugs are loaded in a non-crystalline form (Mehling, Smirnova, Guenther & Neubert, 2009). Accordingly, ibuprofen and ketoprofen-loaded organic aerogel monoliths and beads (corn starch and alginate) showed faster drug dissolution rate than the crystalline drug (Del Gaudio, Auriemma, Mencherini, Porta, Reverchon & Aquino, 2013; Mehling, Smirnova, Guenther & Neubert, 2009).

Aerogels produced in the form of microspheres (Valentin, Molvinger, Quignard & Di Renzo, 2005) are preferred as drug carriers to other aerogel formats, such as monoliths (García-González, Camino-Rey, Alnaief, Zetzl & Smirnova, 2012), granules (Hong, Yoon & Hwang, 2011), powders (Bhagat, Park, Kim, Kim & Han, 2008) or beads (Sarawade, Kim, Hilonga, Quang, Jeon & Kim, 2011). This preference for the microspherical form arises from its high flowability, ease of handling, and improved processing reproducibility coupled to reduced triggering of inflammatory response due to the inherent absence of sharp edges (Hong, Yoon & Hwang, 2011; Radin, Chen & Ducheyne, 2009). Release of active pharmaceutical ingredients (API) from microspheres is a complex process being influenced by various factors that could lead to totally different release profiles. Porous network collapse of aerogel carriers once in contact with aqueous medium notably influences API release. Erosion (by dissolution or hydrolysis) rate depends on the structure and the molecular weight of the polymer, the morphology and size of the carrier, as well as the medium conditions (pH, temperature, enzymes). Polymeric microspheres may erode from the bulk (homogeneous) or from carrier surface (heterogeneous) depending on the relative ratio of water penetration rate to polymer network hydrolysis rate being high or low, respectively, (Rothstein, Federspiel & Little, 2009; von Burkersroda, Schedl & Göpferich, 2002) thus influencing the API release into the environment (Kim & Pack, 2006; O'Donnell & McGinity, 1997). Finally, chemical interactions between the drug and the carrier may also influence the release rate.

There is still a paucity of information about the effect of the polysaccharide source used to prepare microspherical aerogels on their ability to load and to control the release of active substances. Polysaccharide nature may likely influence the aerogel collapse and, subsequently, the mass transport and release profile of the drug contained in the aerogel as well. The aim of this work was to evaluate

the drug loading and release behavior of aerogel microspheres prepared from three polysaccharides (starch, alginate, and pectin) according to an emulsion-gelation method followed by supercritical drying, in order to elucidate their potential as carriers of poorly water soluble drugs for oral administration. Ketoprofen and benzoic acid were loaded into the aerogels by supercritical (sc)CO_2 fluid-assisted impregnation. Silica aerogel microspheres were used as aerogel reference regarding drug loading capacity and release for the sake of comparison. Drug release kinetics from polysaccharide aerogel microspheres were evaluated at gastric and intestinal pH conditions. To the best of our knowledge, this work represents the first systematic study in the fast developing field of the microformulation of supports for drug release using aerogels in the form of microspheres as drug carrier.

2 Materials and methods

2.1 Reagents

Native corn starch (Starch amylo N-460; 52.6% amylose content) was from Roquette (France); alginic acid sodium salt (brown algae origin, G/M ratio of 70/30, MW 403,000 $g \cdot mol^{-1}$) from Sigma Aldrich (Germany); citrus high methoxyl pectin (63-66% degree of esterification, MW 30,000-100,000 $g \cdot mol^{-1}$) and tetramethoxysilane (TMOS, purity of 98%) from Fluka (Germany). Methanol (95%), hydrochloric acid (30%), ammonium hydroxide (25%) and benzoic acid (98%) were from Merck (Germany). Ketoprofen (racemic mixture) was from Chemische Fabrik Kreussler & Co. GmbH (Germany). Ethanol (99.8%) was from Omnilab (Germany) and domestic grade rapeseed oil was purchased from Brökelmann Co. (Germany). Carbon dioxide was supplied by AGA Gas GmbH (Germany).

2.2 Preparation of spherical aerogel microspheres

Aerogel microspheres were obtained applying an emulsion-gelation method followed by supercritical drying. In brief, this method consisted on the preparation of a water-in-oil emulsion (or ethanol-in-oil for silica gel microspheres) followed by the gelation of the dispersed phase (thermal gelation for starch and chemical gelation for alginate, pectin and silica) as previously reported (Alnaief, Alzaitoun, García-González & Smirnova, 2011; Alnaief & Smirnova, 2011; García-González, Uy, Alnaief & Smirnova, 2012). Gel microspheres were isolated through centrifugation and exposed to ethanol for

solvent exchange. Finally, aerogel microspheres were obtained by $scCO_2$-assisted drying. The operating conditions used in this work for the preparation of aerogel microspheres correspond to the ones reported in the literature leading to the highest (or close to the highest) specific surface area values so far for each aerogel case using processing approaches compatible with the intended application (i.e., oral drug delivery). Operating conditions for the processing of the aerogel microspheres were chosen so that mean pore diameters in the range 14-18 nm were obtained in all cases to neglect the effect of this variable in the study. For more detailed instructions on the preparation of the aerogels, please refer to the Appendix A in Supplementary Data section.

2.3 Ketoprofen and benzoic acid supercritical impregnation

Alginate, starch, silica or pectin aerogel microspheres (0.12 g) and active compounds (0.12 g of ketoprofen or benzoic acid) were wrapped separately in filter paper cartridges. Then, a set of two cartridges (one containing microspheres and the other with the drug) were placed in one of the reactors of the apparatus sketched in Figure 1. Control trials (one reactor containing a drug cartridge alone and other containing one cartridge for each aerogel type) were carried out in the two remaining reactors. Experiments were carried out in duplicate. The supercritical impregnation setup used for these experiments not only provides a high-throughput trial program by means of its six reactors arranged in parallel (namely, six experiments can be carried out at the same time), but also improves the reproducibility of the experiments since all trials from the same batch series share the same CO_2 pressurization/depressurization cycle and heat transfer histories. $scCO_2$–assisted impregnation conditions were optimized for ketoprofen (40 ºC, 18.0 MPa) and benzoic acid (55 ºC, 18.0 MPa) following guidelines of solubility of active compounds in $scCO_2$ (Jin, Zhong, Zhang & Li, 2004; Stassi, Bettini, Gazzaniga, Giordano & Schiraldi, 2000). Impregnation times were chosen long enough (24h) to neglect the effect of aerogel microparticle size in the drug loading achieved. Impregnation conditions were kept constant under agitation at 500 rpm.

2.4 Aerogel microsphere characterization

Specific surface area of the bare aerogel microspheres was quantified from low-temperature N_2 adsorption-desorption data (Nova 3000e, Quantachrome, USA). Prior to measurements, samples were

dried for 20 h under vacuum (<1 mPa) at 60ºC for alginate and pectin, 80ºC for starch and 200ºC for silica aerogels. Mean particle size of wet gel microspheres dispersed in ethanol was estimated by means of laser diffraction spectrometry (Beckman Coulter LS1332) and using similar obscuration values (9-11%) for all samples. Morphology of the polysaccharide aerogel microspheres as well as silica microspheres was evaluated using scanning electron microscopy (SEM, Leo Zeiss 1530, Germany). Morphology and physical integrity of aerogels in contact with water were monitored with aid of high-speed camera by depositing 100 picoliter-water droplets on microsphere surfaces (DSA 100M provided by Krüss GmbH, Germany). X-ray diffraction analysis (XRD, Siemens D500 diffractometer equipped with a diffracted beam monochromator, Germany) of the drug-loaded and unloaded microspheres as well as aerogel-plus-drug physical mixtures was carried out using a position-sensitive detector and CuKα radiation within the range of 2° to 40° 2θ and a step size of 0.05º. For determination of drug entrapment efficiency, drug-loaded microspheres (10-50 mg) were dispersed in a known volume of ethanol (sink conditions) and sonicated for 30 min, which was enough for complete drug release. Afterwards the solution was filtered through a 0.22 µm membrane filter (Millipore syringe filter, PTFE, EW-29950-42, Cole-Parmer, USA) and the absorbance at 255 nm (ketoprofen) or 227 nm (benzoic acid) measured (Evolution 300 UV spectrophotometer, Thermo Scientific, USA).

2.5 *In vitro* drug release

Dissolution test was conducted according to the Ph. Eur. guidelines in 0.1 M phosphate buffer saline (PBS, simulated intestinal fluid, pH 6.8) or in 0.1 N HCl (simulated gastric fluid, pH 1.2) solutions using a paddle apparatus (Sotax At7, Allschwil, Switzerland) with a constant agitation speed of 100 rpm at 37 °C. Drug-loaded aerogel microspheres (20-50 mg) were immersed in jars containing 900 mL buffer medium (sink conditions). Samples (2 mL) were withdrawn at determined time intervals and filtered through a 0.22 µm membrane filter. Each drug release test lasted for 24 h and was run in duplicate. Drug concentration was determined by means of UV spectrophotometry, as described in section 2.4.

3 Results and discussion

3.1 Characterization of the drug-loaded aerogel microspheres

Aerogel microspheres were obtained by means of an emulsion-gelation method coupled to supercritical drying, in the form of a powder constituted by non-agglomerated particles of mean particle size in the range of 100 to 550 µm (Table 1) depending on the nature of the precursor. Alginate and silica aerogel microspheres showed the lowest mean particle diameters. Particle sizes of aerogel microspheres are mainly influenced by the gelation process and chemical composition (i.e. gelation mechanism, cross-linker, viscosity of the emulsion, emulsifier used or stirring rate among others) (García-González, Alnaief & Smirnova, 2011; García-González, Uy, Alnaief & Smirnova, 2012). Resulting aerogels showed low density (ρ<0.25 g/cm^3), high porosity (ε>85%) and high specific surfaces area (>100 m^2/g, Table 1). Specific surface areas of pectin and alginate aerogels were much higher than those of starch aerogels, but still significantly lower than that of silica aerogel. The values for the specific surface area depend on the three-dimensional structure of the gel, which is mainly governed by the degree of crosslinking of the gel (García-González, Alnaief & Smirnova, 2011). For starch aerogels, higher specific surface area is accordingly obtained by using a high amylose content starch source that provides a less-ordered amorphous network structure, a gelation temperature high enough to promote full disruption of the original starch granules and a low retrogradation temperature favoring crystal nucleation rather than crystal growth (García-González & Smirnova, 2013; Mehling, Smirnova, Guenther & Neubert, 2009). For Ca-alginate aerogels, an increase in the calcium cross-linker salt concentration and in the alginate concentration leads to an increased degree of cross-linking (egg-box model) and a subsequent increase in the specific surface area obtained (Alnaief, Alzaitoun, García-González & Smirnova, 2011). For pectin aerogels, the choice of the pectin source (with different degrees of esterification) and the gelation mechanism (acidic, thermal or ionic gelation mechanism) largely influences the physical stability and specific surface area of the aerogel (García-González, Carenza, Zeng, Smirnova & Roig, 2012; White, Budarin & Clark, 2010).

SEM images of aerogel microspheres before and after impregnation with ketoprofen and benzoic acid (Fig. 2) revealed no significant morphological changes in the matrix due to drug impregnation via

$scCO_2$ technology. High magnifications (Fig. 2,right) evidenced that drug loading did not affect the nanoporous structure of matrices and no sharp edges typical for drug crystals were observed in any of the ketoprofen or benzoic acid-loaded microspheres.

Production of the aerogel microspheres through emulsion-gelation did not influence the crystallinity of the polysaccharide matrix, as confirmed by XRD analysis (Fig. 3). Moreover, the absence of the characteristic peaks of ketoprofen (6.4, 18.2 and 23.2º 2θ) and benzoic acid (8.2° 2θ) in the respective drug-loaded aerogel microspheres confirmed the absence of drug crystals in the aerogels. Conversely, peaks corresponding to benzoic acid and ketoprofen crystals were observed for the physical mixtures of each drug with ground powders of starch microspheres (Figs. 3d and 3g) prepared at similar mass ratio as in the drug-impregnated aerogels; thus it was discarded that the XRD sensitivity was not high enough to detect the presence of drug crystals in the drug-loaded aerogels. Moreover, control trials with the drugs processed at the same supercritical operating conditions used for drug impregnation in aerogels show neither change in the XRD-pattern with respect to the raw drug nor the appearance of any polymorph of the drug. Amorphisation of the drug loaded in the aerogel matrices should be linked to the impregnation process itself.

Contact angle measurement test by applying a water droplet to the aerogel microsphere surfaces was used to determine their hydrophilicity/hydrophobicity as well as to get an indication of the aerogel structure integrity behavior and morphology in presence of water. Due to the porous structure of aerogel microspheres, water droplet was absorbed into the network and disappeared in few milliseconds (Fig. 4). Afterwards, polymer erosion occurred for aerogels and the structure collapsed at different velocities and intensities depending on the type of polymeric material. Starch microspheres showed more resistance against hydration, whereas silica and pectin microspheres were prone to a rapid collapse of the porous network once in contact to water. Any conclusion can be poorly derived for alginate microspheres based on the sensitivity of the measurement method due to the small diameter of the aerogel, although a sudden material collapse seems to appear just after water deposition (Fig. 4-bottom center). The observed pore collapse is a physical degradation taking place due to mechanical stresses: upon contact with an aqueous medium and due to the liquid/air interfacial surface tension, the open nanoporous structure of hydrophilic aerogels allows the penetration of water

therein resulting in large capillary forces taking place and inducing compression stresses enough to collapse the aerogel network (Hüsing & Schubert, 1998). In general, a more rapid matrix collapse may accelerate drug release (Kim & Pack, 2006; Mathiowitz et al., 1997; Shen, Kipper, Dziadul, Lim & Narasimhan, 2002; Spenlehauer, Vert, Benoit & Boddaert, 1989). Even though erosion is not the only factor influencing the drug release process, this experiment may shed light on detection of erosion during the drug dissolution process and to what extent it may play a role in releasing drug molecules into the medium.

3.2 Drug entrapment efficiency

Ketoprofen and benzoic acid were loaded into the aerogels by supercritical impregnation. The preference for supercritical impregnation of aerogels rather than other drug loading techniques (e.g., before gelation or during solvent exchange) stems from the poor solubility in water and good solubility in $scCO_2$ of these drugs, thus leading to higher drug loadings of the aerogels in the former case (García-González, Alnaief & Smirnova, 2011). As a rule-of-thumb, the highest drug loading capacities of aerogels are usually correlated with the largest specific surface area. Accordingly, silica aerogel microparticles had the highest drug loading for both ketoprofen and benzoic acid, among the four types of microspheres tested (Table 1). Nevertheless, drug loading of alginate, starch and pectin microparticles did not obey the same tendency and was also dependent on the drug to be loaded. Moreover, starch aerogel microspheres were more prone to adsorb drug molecules since they presented the highest specific loading capacity for ketoprofen among the aerogels tested. These findings suggest that, apart from specific surface area, other factors such as surface chemistry and API-aerogel carrier interaction may also influence the drug adsorption when using different sources of polymer matrices and thus determining the percentage of drug loading in the aerogel. Specific loading values for ketoprofen were lower than those expected for a monolayer coverage ($1.7\text{-}2.1\times10^{-3}$ g/m^2) in all cases. Ketoprofen adsorption coverages in the aerogels below the monolayer coverage might be explained by surface hydroxyl group density in the aerogel and the competitive physisorption of CO_2 molecules during the supercritical fluid-assisted impregnation process (Gorle, Smirnova & Arlt, 2010; Tripp & Combes, 1998). Ketoprofen interacts with hydroxyl groups in the aerogel by hydrogen bonding through the carbonyl and carboxyl groups present in this drug molecule (Lozano & Martínez,

2006). The density of OH groups in starch aerogels is estimated to be 4–10 times higher than that of silica aerogels (García-González, Camino-Rey, Alnaief, Zetzl & Smirnova, 2012). Starch is also the only polysaccharide used in this study without acid groups in its molecular structure, thus avoiding the electrostatic repulsions of the drug molecules that may take place for the other polysaccharides. Stronger chemical interaction between the adsorbed drug molecules and the starch matrices might be thus behind the observed behavior of high ketoprofen specific loadings compared to the other aerogels. Moreover, starch (mainly amylose) can adopt helical structures with a hydrophobic cavity able to host drugs, such as benzoic acid (Uchino, Tozuka, Oguchi & Yamamoto, 2002) and recently reported for ketoprofen (Messner, Häusler & Loftsson, 2012), leading to the formation of inclusion complexes, which should contribute to greater drug uptakes. Namely, benzoic acid was reported to induce changes in the amylose helical structure to form inclusion complexes of amylose with the drug by using the sealed-heating process (Uchino, Tozuka, Oguchi & Yamamoto, 2002). This processing technique stems from the ability of evaporation of the drug at a given temperature to access the amylose helical structure and form the inclusion complex, observed through the appearance of XRD peaks at 6.8, 12.9, 18.0º 2θ in the case of benzoic acid. Analogously, the supercritical impregnation process might be a low-temperature alternative to the sealed-heating process able to incorporate the benzoic acid in the helical amylose cavities due to the inherently low viscosity and high diffusivity of the supercritical solutions containing the drug. Evidences of inclusion formation by XRD in the benzoic acid-loaded starch aerogel cannot be confirmed in Fig 3e due to the low degree of crystallinity of the sample arising from its high amylose content and low hydration (Cheetham & Tao, 1998). Nevertheless, the presence of an incipient peak at 18.0º 2θ (as indicated by an asterisk in the figure), main diffraction peak of benzoic acid–7_1-helix structure amylose inclusion complex, seems to indicate the formation of an inclusion complex between benzoic acid and the starch (amylose) matrix. For the other aerogels loaded with benzoic acid, the specific loadings lies in the region of 2-4 × 10^{-4} g benzoic acid/m^2 where the absence of drug crystals was previously reported (Gorle, Smirnova & Arlt, 2010). Overall, benzoic acid loading and drug entrapment efficiency values were much higher than ketoprofen loading ones regardless of the polymer matrix considered. This can be explained by the much lower molecular weight of benzoic acid (122.12 g/mol for benzoic acid and 254.28 g/mol for ketoprofen) as well as the

configuration of benzoic acid molecule (flat molecule: 0.50 nm×0.72 nm) compared to ketoprofen molecule (3D-molecule: 0.50 nm×1.22 nm×0.45nm).

3.3 Release of ketoprofen and benzoic acid

For drug delivery systems composed of biodegradable and water-soluble organic materials, three important factors –diffusion, dissolution and polymeric matrix erosion upon degradation – can be involved in the drug release process. *In vitro* cumulative release profiles of ketoprofen from different types of polysaccharide aerogel microspheres in pH 6.8 buffer solution are shown in Fig. 5. Similar patterns were observed in all cases, i.e. an initially fast dissolution rate during the first two hours was followed by a sustained release up to reaching a plateau. During these two hours, alginate and pectin microspheres released *ca.* 80% of the total ketoprofen impregnated, whereas starch microspheres released only 54.5%. The faster release rate of alginate and pectin microspheres in comparison to starch ones may stem from the matrix interaction with the aqueous medium and also from the weaker drug-matrix interactions. Pectin (Vaclavik & Christian, 2007) and alginate (Berger, Ludwig & Wielich, 1953) are proved to be highly hydrophilic leading to rapid collapse of the aerogel porous network in aqueous medium; thus, amorphous drug molecules can be released to the buffer solution. Besides, the different drug release rate from alginate and pectin microspheres in comparison to that from starch microparticles may stem from the higher specific surface areas of the former ones (Table 1). The slightly incomplete release observed for pectin microspheres (82.02 % at 1440 min) compared to alginate ones, can be explained by the combination of lower specific surface area, larger particle size of pectin and drug-matrix chemical interactions.

The release of ketoprofen contained in silica aerogel microspheres, a non-biodegradable polymeric matrix, showed a prompt drug release (60% in the first 60 min) followed by a slow release of 2% within the next 23 h (Fig. 5). The high specific surface area of silica aerogels facilitates the release of larger amounts of ketoprofen as a burst compared to the release of the drug from the polysaccharide aerogels. The non-degradability of silica under the release conditions coupled to the dramatic blockage of the drug in the inner core of the silica micropheres due to the porous network collapse observed for these aerogels once in contact with water may hinder the release by diffusion of the remaining ketoprofen to the surrounding medium.

Three mathematical models (Korsmeyer-Peppas model (Eq. (1)), first-order model (Eq. (2)) and Gallagher-Corrigan model (Eq(3))) emphasizing on different release phenomena were used to fit the drug release profile with the aid of Origin 8 software (Balcerzak & Mucha, 2010; Dash, Murthy, Nath & Chowdhury, 2010):

$$F = k \cdot t^{n} \qquad \text{Eq. (1)}$$

$$F = F_{max}\left[1 - \exp(-k_1 \cdot t)\right] \qquad \text{Eq. (2)}$$

$$F = F_{max}\left[1 - \exp(-k_1 t)\right] + (F_{max} - F_B)\left[\frac{\exp(k_2 \cdot t - k_2 \cdot t_{2max})}{1 + \exp(k_2 \cdot t - k_2 \cdot t_{2max})}\right] \qquad \text{Eq. (3)}$$

where F is the fraction (in percentage) of drug released at a certain time t, F_{max} stands for the maximum fraction of drug released during the total time period, and k_1 and k_2 are the first order kinetic coefficient and the kinetic coefficient for the second stage in min^{-1}, respectively. In Eq. (1) n denotes the release exponent, while F_B in Eq. (3) indicates the drug fraction released in the first release stage. The criterion of only validating a correlation when $R^2 > 0.98$ was adopted.

The Gallagher-Corrigan model fitted ketoprofen release from pectin aerogel microparticles ($R^2 > 0.999$, Table 2). This model fitting denotes a two-phase drug release profile, incorporating an initial burst release (phase I) followed by a slower polymeric matrix bulk degradation (erosion)-controlled release phase (phase II) (Gallagher & Corrigan, 2000). During the phase I, a fraction of 25.0% of the total non-crystalline drug (F_B) easily dissolved once the matrix comes into contact with the surrounding aqueous medium, which meant that this fraction of drug was the most accessible one, being only physically adsorbed to the external surface or in large pores of the aerogel matrix. Ketoprofen fraction entrapped in the pectin microspheres represents 56.6% of drug molecules (F_{max}-F_B) and is only released out in the phase II after pectin aerogel matrix degradation. Moreover, pectin microspheres provided a rapid release profile with a significantly higher simulated degradation kinetic coefficient (k_2=0.093 min^{-1}) compared to diffusion kinetic coefficient (k_1=0.023 min^{-1}). This suggests a rapid bulk degradation process, which is attributed to the high hydrophilicity and chemical instability of pectin aerogels in pH 6.8 buffer solution.

For ketoprofen release from alginate aerogel microspheres, both Korsmeyer-Peppas and Gallagher-Corrigan models showed comparable fitting wellness ($R^2 > 0.99$, Table 2). In this case, the diffusion

kinetic coefficient (k_1=0.026 min^{-1}) was higher than the degradation kinetic coefficient (k_2=0.010 min^{-1}), suggesting that drug release is likely diffusion-controlled assisted by polysaccharide erosion. The *n* value of Korsmeyer-Peppas model below 0.43, an extreme value for spherical dosage forms (Ritger & Peppas, 1987), suggests the initial diffusion process of ketoprofen following the Fickian diffusion mechanism and endorses the diffusion-control hypothesis.

First order kinetics was the best model fitting ketoprofen release from starch aerogel microspheres (R^2=0.987) (Table 2). The wellness of the first order model fitting suggests that drug dissolution from starch aerogel microspheres is the governing factor of ketoprofen release where the solid matrix morphology remains intact during the dissolution process. Gallagher Corrigan model, with an excellent correlation and a significantly low k_2 value, and Korsmeyer-Peppas model, with a n-value of 0.40, endorsed a first-order model following the Fickian release. A prompt drug release from starch microspheres resulted in 53% of the total loaded drug dissolved in neutral medium in the first 60 min, followed by an increment of 3% in release rate within 24 h. Drug remaining in the starch aerogel may be forming an insoluble inclusion complex in the aerogel matrix.

Release behavior of ketoprofen from aerogel microspheres was also investigated at acidic conditions (pH 1.2, gastric pH conditions), in order to determine their potential to promote fast release in the gastric environment (Figs. 6 and 7). Ketoprofen, as well many other non-steroidal anti-inflammatory drugs (NSAIDs) is characterized by a pH-dependent solubility profile due to their weak acid character (Sheng, Kasim, Chandrasekharan & Amidon, 2006). Thus, ketoprofen crystals (pKa 4.4-4.8) exhibit low solubility and dissolution rate under acidic conditions. Moreover, cross-linked pectin and alginate microspheres are resistant to acidic conditions and are not able to dissolve in aqueous media, likely influencing the release behavior of the drug incorporated to these carriers (Del Gaudio, Colombo, Colombo, Russo & Sonvico, 2005; Sriamornsak, 2003). The kinetic analysis of the ketoprofen release from alginate and pectin microsphere aerogels is summarized in Table 3.

In contrast to drug release profiles at pH 6.8, alginate microspheres accelerated ketoprofen release at simulated gastric pH conditions (Fig. 6), which is consistent with previous reports (Radin, Chen & Ducheyne, 2009). pK_a values of both alginate (1.5-3.5) and ketoprofen (4.4-4.8) are in the 1.2-6.8 pH range leading to different ionic forms of the drug and the carrier in PBS and simulated gastric fluid

conditions. Besides, non-crystalline drug molecules located in aerogel microspheres may require much less time for dissolution than pure ketoprofen crystals (Sheng, Kasim, Chandrasekharan & Amidon, 2006). As denoted by the Korsmeyer-Peppas model, ketoprofen release from alginate aerogel microspheres at pH 1.2 mainly follows a diffusion profile similar to that obtained at pH 6.8, but with a higher value of the diffusion coefficient at pH 1.2.

Ketoprofen release from pectin microspheres at pH 1.2 (Fig. 7) was slower than at pH 6.8 during the first 2 h, although again faster than the profile recorded for the free crystalline drug. After 2 h, drug release rate at acidic conditions exceeded the release rate at neutral conditions, finally reaching 98% drug released (compared to 80% released at pH 6.8). This finding can be well explained by the pectin matrix properties. The initial slower release rate at pH 1.2 is due to the lower swelling ratio of the pectin matrix at acidic conditions, due to the high proportion of unionized carboxylic groups. After a certain time period, the swelling effect decreases likely due to matrix degradation (Sriamornsak, 2003) and drug-pectin interaction should govern the drug release. Due to the small particle size of the aerogel microspheres, swelling and water uptake studies were not able to be carried out to confirm this effect, in contrast to other studies with small aerogel monoliths or gel beads both falling in the size range of few milimeters (Betz, García-González, Subrahmanyam, Smirnova & Kulozik, 2012; Del Gaudio, Colombo, Colombo, Russo & Sonvico, 2005), which can be both weighed or measured in a straightforward way. This behavior of pectin supports the final faster release rate of ketoprofen compared to that observed at pH 6.8 conditions. Accordingly, the kinetic coefficient obtained for pectin aerogels at pH 1.2 (k_2=0.113 min^{-1}) by the Gallagher Corrigan model is higher than that at pH 6.8 (k_2=0.093 min^{-1}). Moreover, F_B value at pH 1.2 (86.81%) is much higher than at pH 6.8 (24.98%), which is ascribed to the weaker ketoprofen-pectin interaction at pH 1.2 with respect to pH 6.8 due to the change in the ionic forms of both ketoprofen and pectin at these two pH conditions.

The release behavior from starch aerogel microspheres of ketoprofen was compared to that of benzoic acid. Both drugs have similar chemical functionalities and dissociation constant values (pKa 4.2 for benzoic acid), but different molecular weights and dimensions. Moreover, the presence benzoic acid-amylose inclusion complexes may influence on the release behavior of the drug. Starch microspheres loaded with benzoic acid showed the same release pattern at intestinal environment (pH 6.8) (Fig. 8)

as with ketoprofen, i.e. a burst release followed by a sustained release. Starch microspheres possess a strong hydrophilic character and water uptake is considered to be the main controlling mechanism in the initial burst period and the release period is complete within 30 min, as also reported by other researchers (Atyabi, Manoochehri, Moghadam & Dinarvand, 2006). The dissolution profile of pure benzoic acid was similar to that of the same drug loaded in the aerogel, but showing an initially slower release rate according to the k_1 parameter from the first order release model fitting (0.160 min^{-1} for benzoic acid in the starch aerogels and 0.071 min^{-1} for pure benzoic acid) likely related to the amorphous state of the drug in the aerogel. However, with regard to the final benzoic acid release percentage, only 63% of the total loading content from starch microspheres was released into the aqueous medium whereas 90% pure benzoic acid was dissolved in the first two hours. This could be the consequence of closed trapping of solute drug molecules into the starch polymeric network by formation of chemical bonds (carboxylic group of benzoic acid with hydroxyl group of starch matrix) (Vyas & Jain, 1992) and the presence of amylose-benzoic acid inclusion complexes. Release of benzoic from starch aerogel microspheres was faster than that of ketoprofen with the same aerogel carrier. The difference in dissolution behavior between ketoprofen and benzoic acid may stem from the smaller dimension of benzoic acid, which makes it easier diffusing from starch polymeric network, and different drug-matrix interactions.

4 Conclusions

Different types of biodegradable polysaccharide-based aerogel microspheres including alginate, pectin and starch were evaluated regarding their feasibility to be used as drug carriers for life science applications. The processing method used provides a suitable morphology of the micronized carrier with improved product reproducibility, an amorphous drug-loaded delivery system with loadings in the range of 11-24 wt.%, and an expected good physicochemical stability upon storage because of the dry solid format of the formulation. Drug loading capacity was dependent on the specific surface area and surface chemistry of the aerogels as well as on the drug-aerogel matrix chemical interaction. Material hydrophilicity tests with a high-speed camera were suitable to unveil different erosion mechanism of the aerogel in contact with aqueous medium depending on the aerogel origin (fast erosion for pectin and alginate and slow erosion for starch aerogels). Release of ketoprofen from

alginate and pectin aerogel particles was sensitive to pH values of the aqueous medium (pH 1.2 or 6.8). Namely, alginate aerogel microspheres showed accelerated drug release behavior at simulated gastric pH conditions favoring ketoprofen dissolution at this point. The release profiles of the polysaccharide aerogel microspheres are governed by one or two release mechanisms depending on the aerogel matrix. This different drug release behavior observed for polysaccharide aerogel matrices opens up the possibility of using these nanostructured materials as carriers for developing tailor-made drug release profiles for oral applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version.

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Table 1. Physical properties and drug loading capacities for aerogel microspheres.

Aerogel matrix	Mean particle size [μm][a]	Specific surface area [m^2/g][b]	Drug loading [wt.%]		Drug entrapment efficiency [%][c]		Specific loading [g/m^2]	
			Ketoprofen	Benzoic acid	Ketoprofen	Benzoic acid	Ketoprofen	Benzoic acid
Silica	155±7	1000±50	15.38±1.80	23.77±0.12	18.18±2.51	31.18±0.21	$1.54\times10^{-4}\pm1.80\times10^{-5}$	$2.38\times10^{-4}\pm6.50\times10^{-6}$
Alginate	116±6	524±26.4	11.83±0.61	18.92±1.32	13.42±0.78	23.33±2.01	$2.26\times10^{-4}\pm1.17\times10^{-5}$	$3.61\times10^{-4}\pm2.51\times10^{-5}$
Pectin	498±1	397±19.9	14.01±3.84	14.66±1.24	16.29±5.19	17.18±1.70	$3.53\times10^{-4}\pm9.66\times10^{-5}$	$3.69\times10^{-4}\pm3.15\times10^{-5}$
Starch	519±4	127±6.4	12.84±0.92	21.54±2.04	14.73±1.21	27.45±3.31	$10.1\times10^{-4}\pm7.24\times10^{-5}$	$17.0\times10^{-4}\pm1.61\times10^{-4}$

[a] Obtained prior to supercritical drying. A particle size decrease of 5-10% is expected after drying

[b] Obtained prior to supercritical impregnation

[c] Note that the remaining drug fraction is recovered in the form of dry powder in the drug cartridge and can be virtually reused for ulterior trials

Table 2. Kinetic fitting parameters of the ketoprofen release from drug-loaded aerogel microparticles in pH 6.8 buffer solution. Bold values denote the best-fit parameters for each ketoprofen-aerogel system

Samples	First Order			Korsmeyer-Peppas			Gallagher Corrigan					
	F_{max} (%)	k_1(min^{-1})	R^2	k(min^{-n})	n	R^2	F_B (%)	k_1(min^{-1})	F_{max} (%)	t_{2max}(min)	k_2(min^{-1})	R^2
Pectin	81.65	0.032	0.985	3.48	0.77	0.983	**24.98**	**0.023**	**81.57**	**22.87**	**0.093**	**0.9995**
Alginate	89.99	0.028	0.923	**12.86**	**0.40**	**0.998**	**50.74**	**0.026**	**97.08**	**42.70**	**0.010**	**0.998**
Starch	**54.90**	**0.075**	**0.987**	12.18	0.40	0.953	**47.96**	**0.068**	**57.21**	**-306.3**[a]	**0.002**	**0.9991**

[a] This unrealistic negative value of t_{2max} is likely a numerical incertitude problem arising from the very low value of k_2.

Table 3. Kinetic analysis of the ketoprofen release from drug-loaded aerogel microparticles in pH 1.2 acidic environment. Bold values denote the best-fit parameters for each ketoprofen-aerogel system.

Samples	First Order			Korsmeyer-Peppas			Gallagher Corrigan					
	F_{max} (%)	k_1(min^{-1})	R^2	k(min^{-n})	n	R^2	F_B (%)	k_1(min^{-1})	F_{max} (%)	t_{2max}(min)	k_2(min^{-1})	R^2
Pectin	95.51	0.014	0.996	1.38	0.93	0.991	**86.81**	**0.015**	**92.90**	**373.76**	**0.113**	**0.997**
Alginate	92.85	0.051	0.949	**16.66**	**0.41**	**0.967**	79.55	0.031	96.12	35.50	-0.127	0.991

Graphical abstract

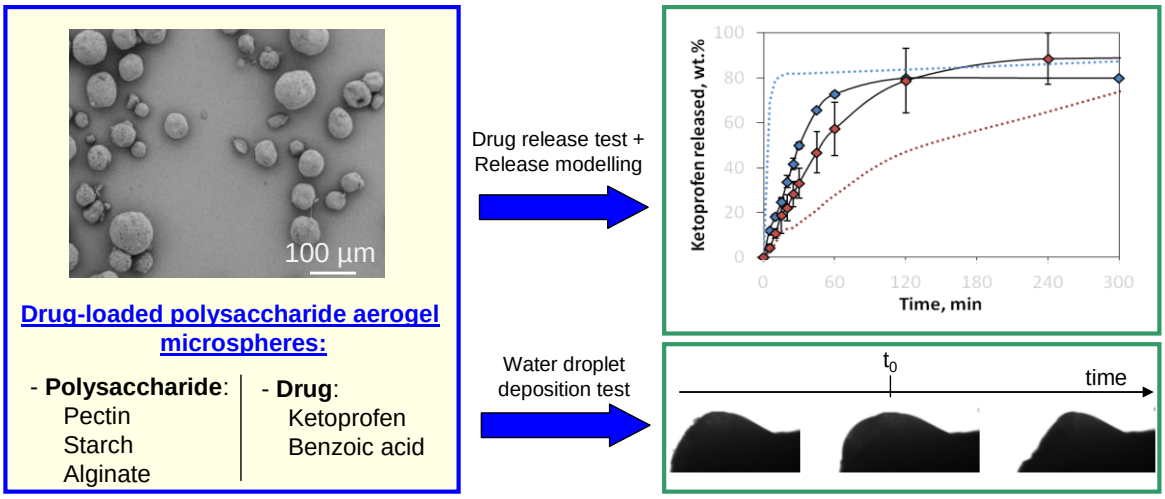


Figure Captions

Fig. 1. Schematic diagram of supercritical CO_2 impregnation equipment

Fig. 2. SEM images of (a) pure alginate aerogel microspheres, (b) ketoprofen-loaded alginate microspheres, and (c) benzoic acid-loaded alginate microspheres. Scale bars represent either 2 µm (left) or 200 nm (right). Alginate microspheres were herein shown as representative examples of the polysaccharide aerogels studied.

Fig. 3. X-ray diffraction patterns for: ① Benzoic acid case study: (a) pure benzoic acid, (b) raw starch material, (c) unloaded starch aerogel microspheres, (d) physical mixture of starch aerogel microsphere with benzoic acid (15 wt.%), and (e) benzoic acid-loaded starch aerogel microspheres. ② Ketoprofen case study: (f) pure ketoprofen, (g) physical mixture of starch aerogel microsphere with ketoprofen (15 wt.%), and (h) ketoprofen-loaded starch aerogel microspheres. Asterisk symbol indicates the main diffraction peak of benzoic acid–7_1-helix structure amylose inclusion complex.

Fig. 4. Contact angle measurement test of starch, silica, pectin and alginate aerogel microspheres (in order from top to bottom). Pictures were taken before (left), at (center) and after (right) dosing a 100 picoliter water droplet. The stop time of the test was selected when no further structure alteration was noticeable and corresponds to the so-called *after deposition test* in the figure. Projections of the contour lines of the particles before water contact are added in the pictures after water contact (dashed red lines) to facilitate the visual inspection of the water drop test.

Fig. 5. *In vitro* release profiles at pH 6.8 (0.1 M phosphate buffer saline solution) of ketoprofen from starch (squares), alginate (diamonds), pectin (circles) and silica (pluses) aerogel microspheres (in solid lines). The dissolution pattern of the same amount of free drug (triangles and dotted line) is also plotted for comparison purposes.

Fig. 6. *In vitro* release profiles of ketoprofen from alginate aerogel microspheres at two different pH media (solid lines). Release studies of the aerogels were carried out at 37ºC and pH 1.2 (0.1 N HCl, dark

25 diamonds) and 6.8 (0.1 M phosphate buffer saline solution, blank diamonds). Release profiles of the raw
26 ketoprofen (dotted lines) are also plotted for the sake of comparison (dark triangles for pH 1.2 and blank
27 triangles for pH 6.8).

28 **Fig. 7**. *In vitro* release profiles of ketoprofen from pectin aerogel microspheres at two different pH media
29 (solid lines). Release studies of the aerogels were carried out at 37ºC and pH 1.2 (0.1 N HCl, dark circles)
30 and 6.8 (0.1 M phosphate buffer saline solution; blank circles). Release profiles of the raw ketoprofen
31 (dotted lines) are also plotted for the sake of comparison (dark triangles for pH 1.2 and blank triangles
32 for pH 6.8).

33 **Fig. 8.** Release profiles for starch aerogel particles loaded with benzoic acid (black squares) and
34 ketoprofen (white squares) at 37ºC and pH 6.8 (0.1 M phosphate buffer saline solution) (in solid lines).
35 Release profiles of the raw ketoprofen (triangles) and benzoic acid (crosses) are also plotted (in dotted
36 lines) for the sake of comparison.

Figure(s)

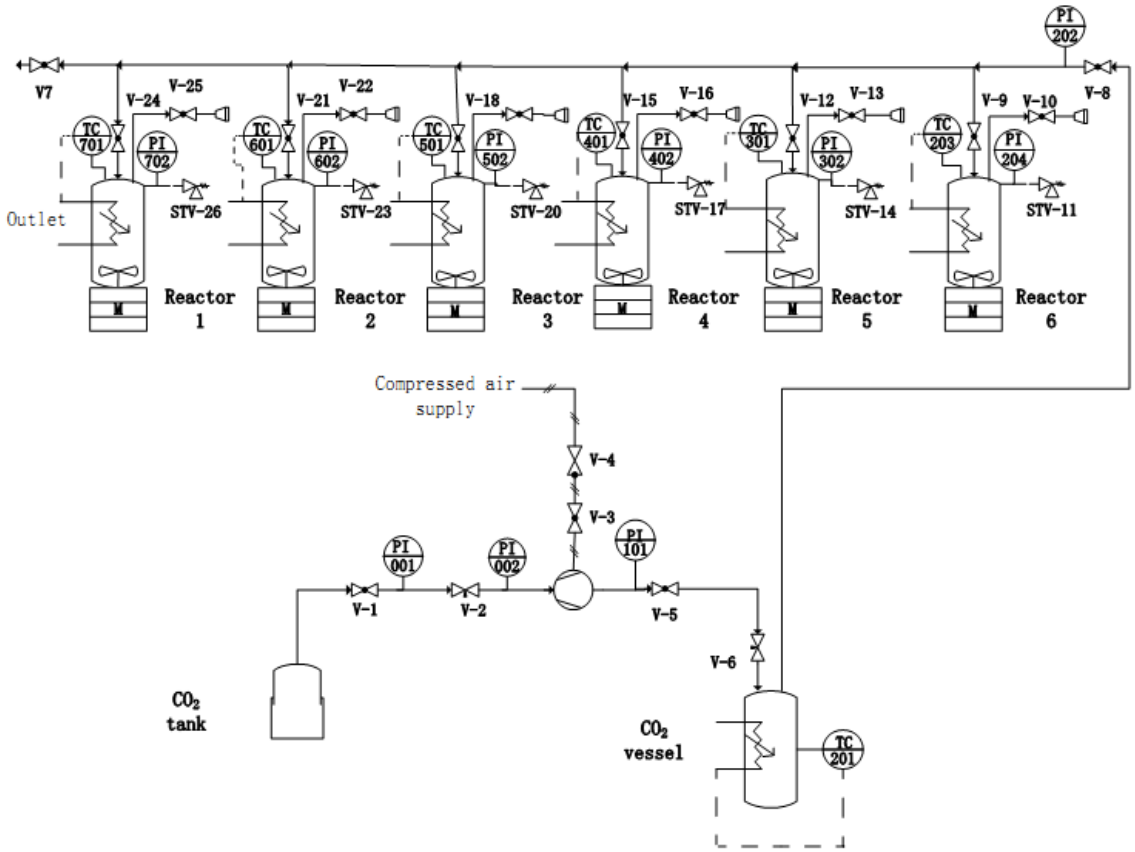


FIGURE 1

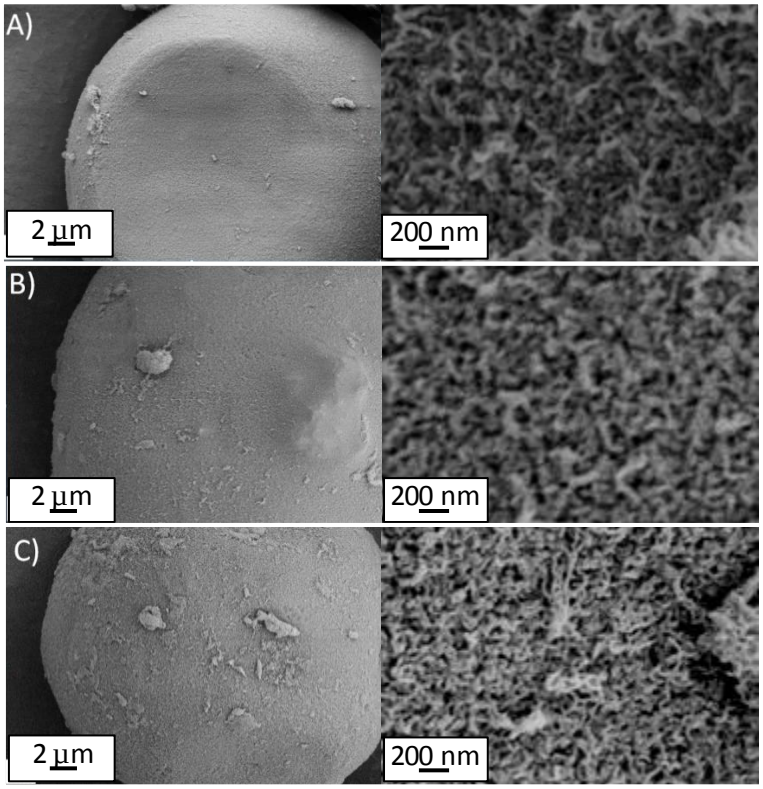
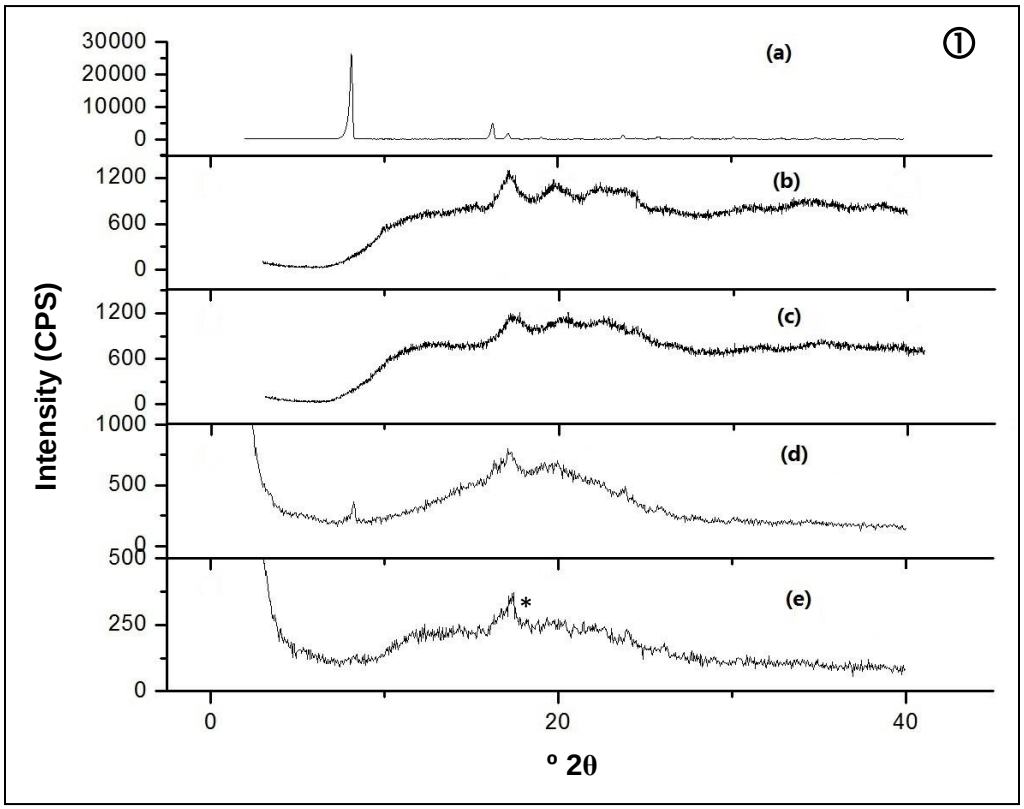


FIGURE 2



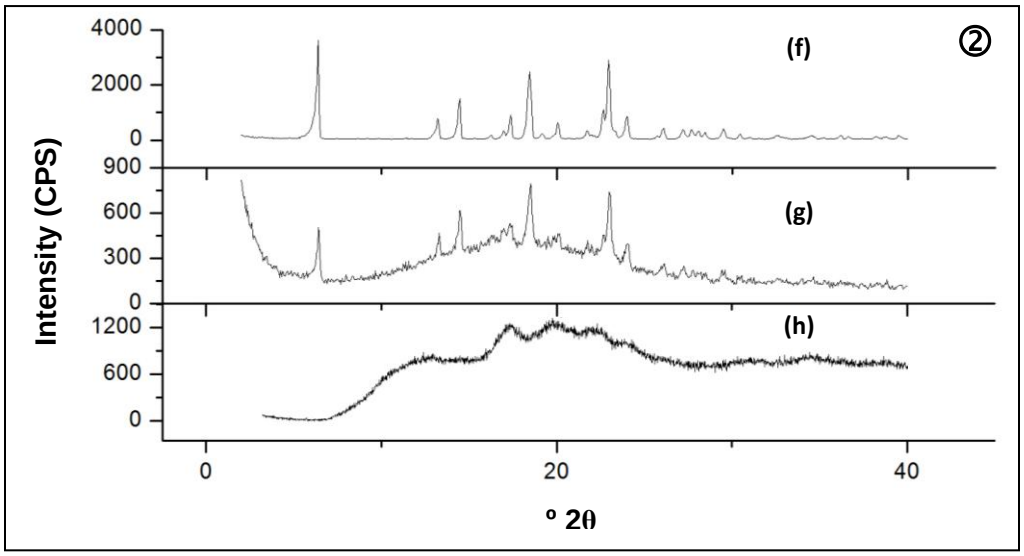


FIGURE 3

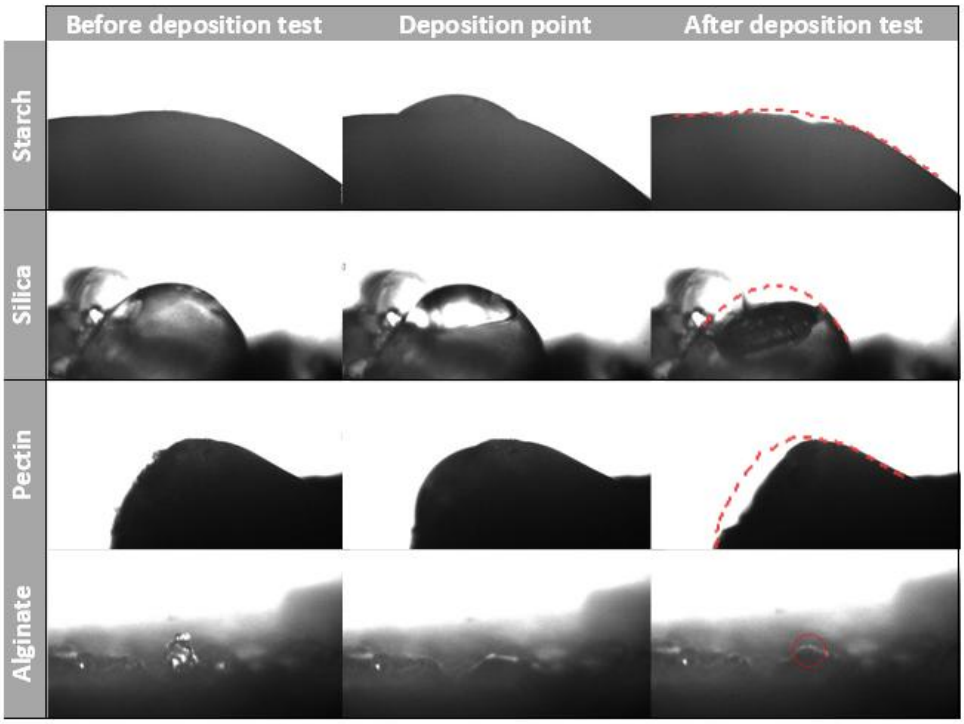

Before deposition test
Deposition point
After deposition test
Starch
Silica
Pectin
Alginate

FIGURE 4

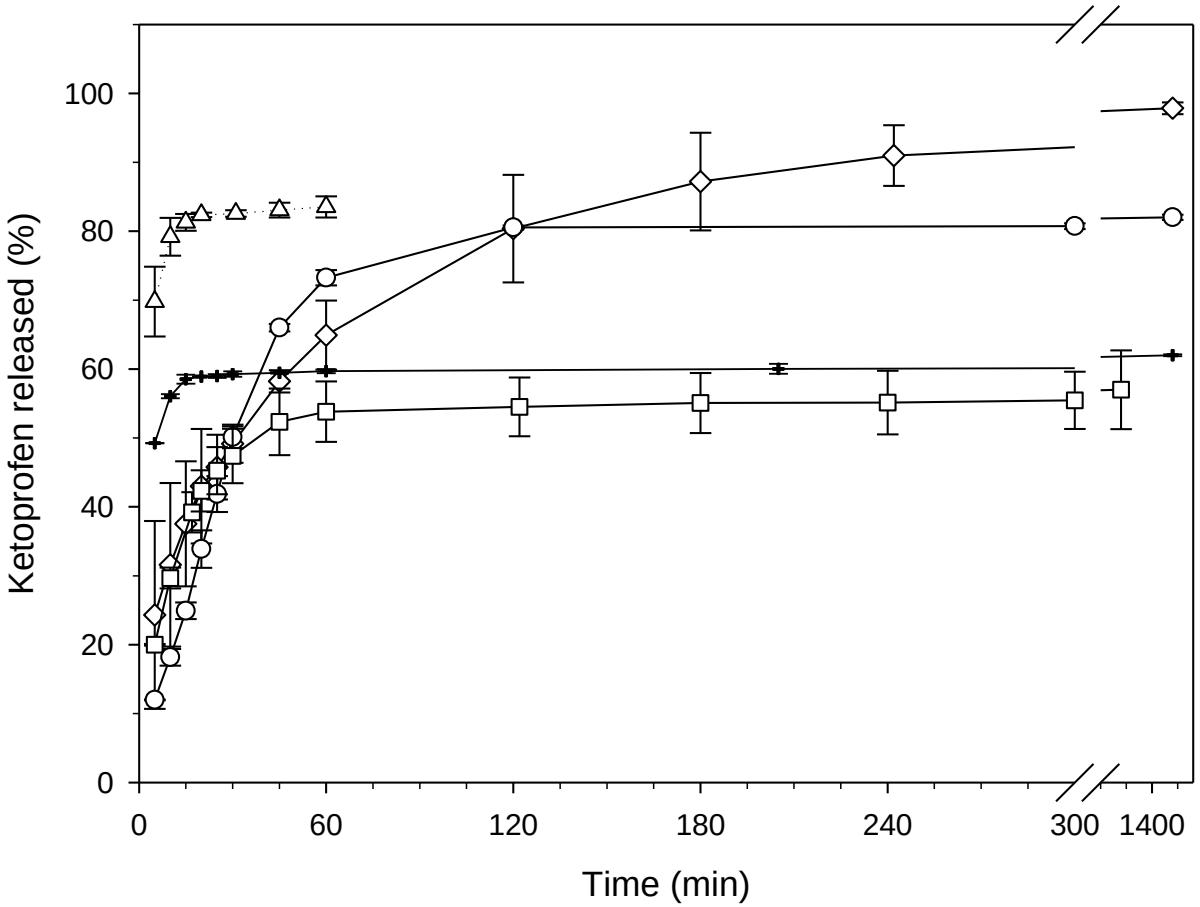

Ketoprofen released (%)
100
80
60
40
20
0
0
60
120
180
240
300
1400
Time (min)

FIGURE 5

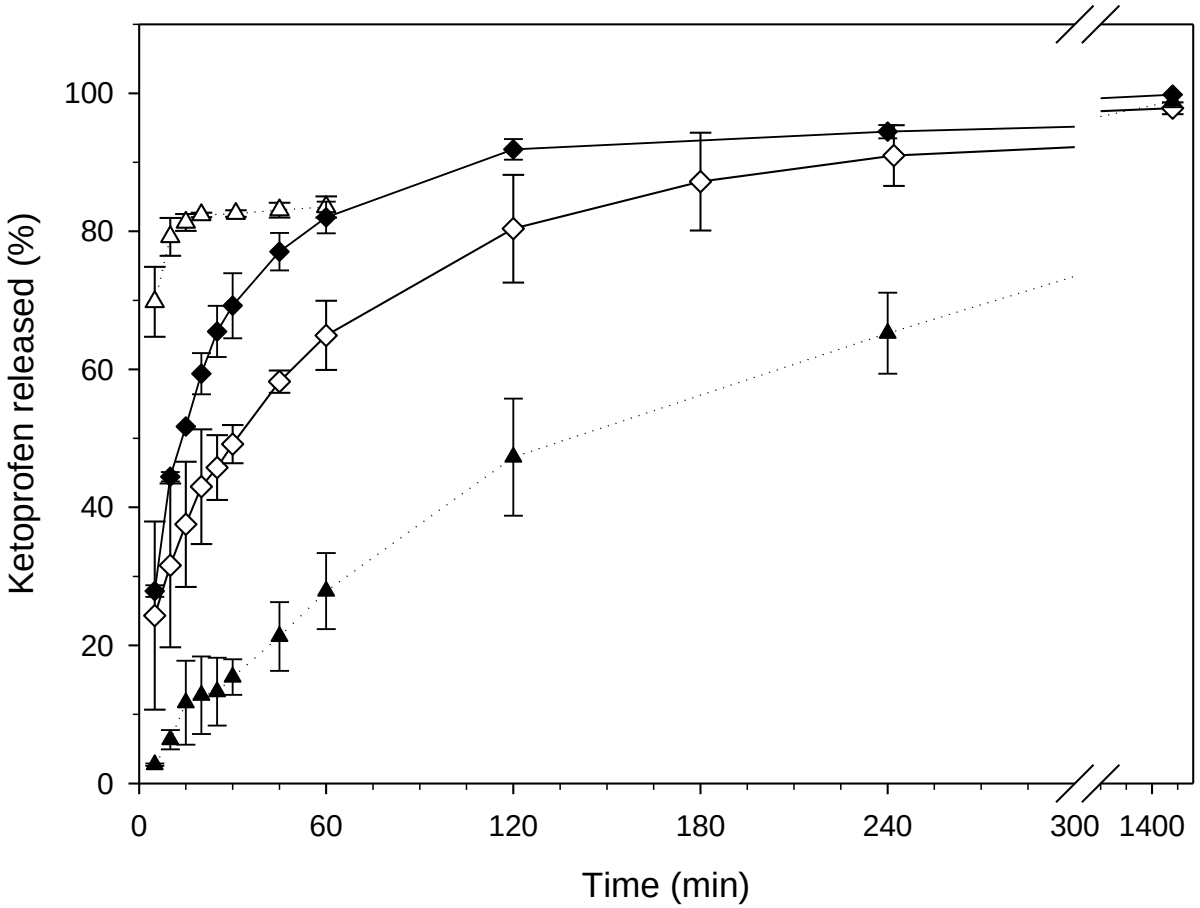

Ketoprofen released (%)
100
80
60
40
20
0
0
60
120
180
240
300
1400
Time (min)

FIGURE 6

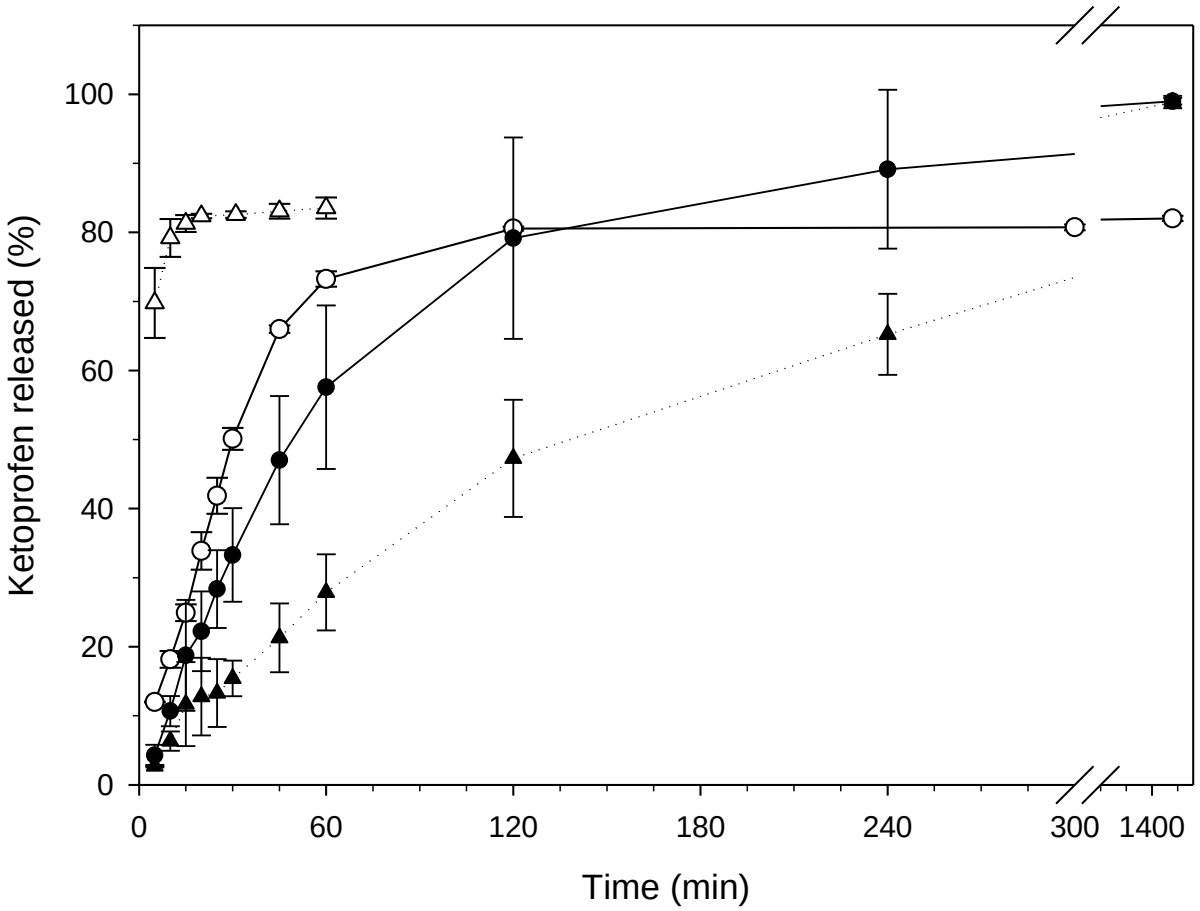

Ketoprofen released (%)
100
80
60
40
20
0
0
60
120
180
240
300
1400
Time (min)

FIGURE 7

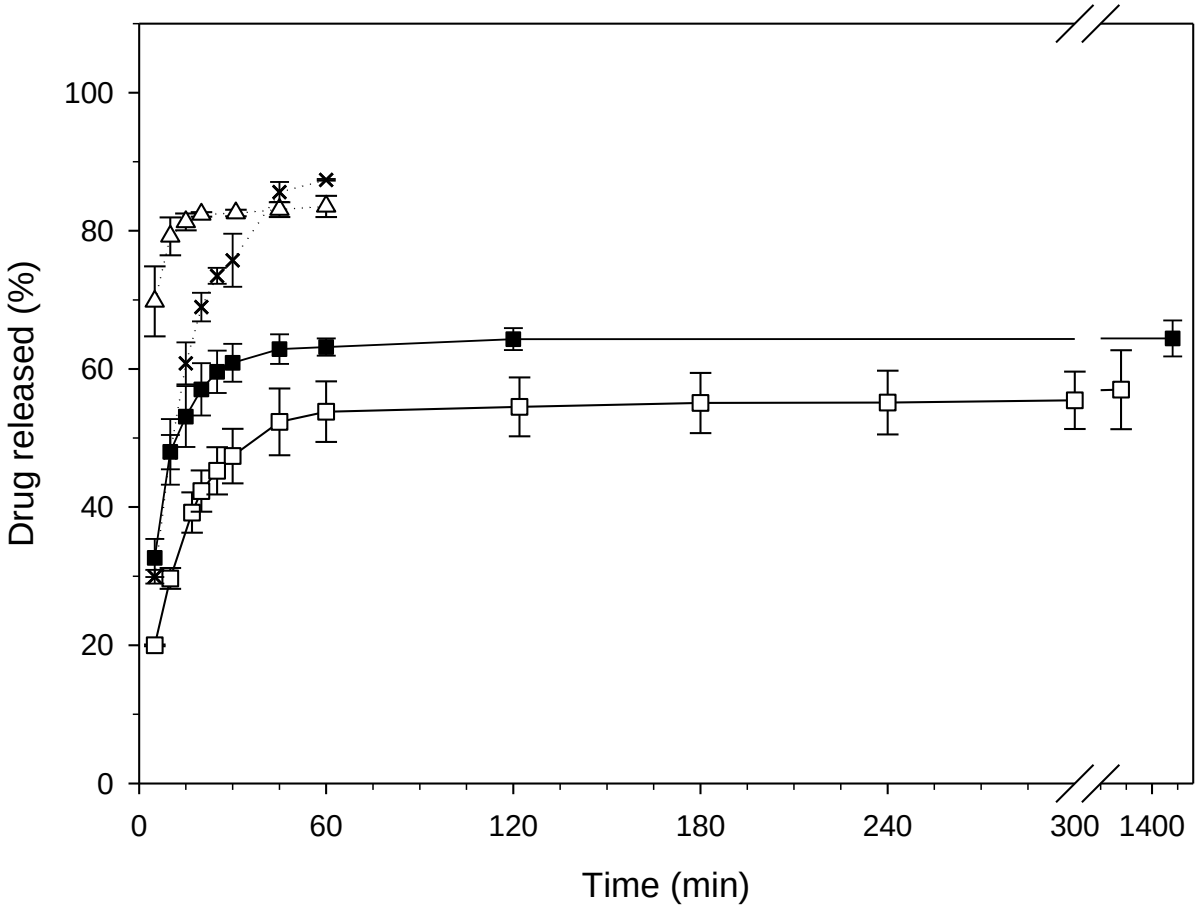

Drug released (%)
100
80
60
40
20
0
0
60
120
180
240
300
1400
Time (min)

FIGURE 8

SUPPLEMENTARY DATA

A. Preparation of spherical aerogel microspheres [1]

A.1 Pectin aerogel [2]

Aerogel microspheres were obtained by stirring (500 rpm) an aqueous phase containing 6% (w/w) of pectin with the corresponding amount of canola oil to get a 3:1 (v/v) oil:pectin solution emulsion. Ethanol (50 wt% with respect to water content) was then added and the mixture was heated to 313 K for 30 min under agitation (500 rpm). Then, the system was cooled down to room temperature and continuously stirred at 2000 rpm. The dispersion was centrifuged to separate the pectin microparticles from the oil phase, immersed in 99.8% (v/v) EtOH and stored at room temperature during 24 h for aging. After a second solvent exchange to ethanol, the pectin gel was supercritically dried at 318 K, 11.0-12.0 MPa and CO_2 flow of 2-4 $NL \cdot min^{-1}$ during 4 h.

A.2 Alginate aerogel [3]

A 2 % (w/w) alginate aqueous solution-in-paraffin oil emulsion (2:1 (v/v) oil:alginate solution) was prepared upon stirring (1000 rpm) at room temperature for 15 min. Prior to the emulsification, $CaCO_3$ was added to the alginate solution (Ca^{2+}/alginate=7.3 wt%) and Span 80 surfactant (surfactant:oil volume ratio=1:99) was added to the oil phase. Gelation of the dispersed phase took place through the internal setting method by the addition of glacial acetic acid (acid-to-Ca^{2+} molar ratio=3.5). Gel microspheres were isolated through centrifugation and exposed to ethanol in a sequential solvent exchange (ethanol:water volume ratio 10:90, 30:70, 50:50, 70:30, 90:10, 99.99:0.01). Finally, aerogel microspheres were obtained by $scCO_2$-assisted drying (318 K, 11.0-12.0 MPa, 2-4 $NLCO_2 \cdot min^{-1}$, 4 h).

A.3 Starch aerogel [4]

A 2:1 volume phase ratio of a 15 % (w/w) corn starch dispersion in the corresponding amount of vegetable oil was prepared. The emulsion was heated in an autoclave to 393 K upon stirring (500 rpm) for 20 min. The emulsion was cooled down to room temperature while stirring at 2000 rpm. Then, the particles were centrifuged to remove the oil and water phase, soaked in ethanol and then stored at 277 K for retrogradation during 48h. After a second solvent exchange to ethanol, the starch gel was supercritically dried at 318 K, 11.0-12.0 MPa and CO_2 flow of 2-4 $NL \cdot min^{-1}$ during 4 h.

A.4 Silica aerogel [5,6]

The sol (dispersed phase) was prepared in two steps: 1) Preparation of a TMOS:MeOH:water:HCl mixture with a molar ratio of $1:2.4:1.3:10^{-5}$ and dilution in ethanol; 2) Addition of water and ammonia solution to obtain a TMOS:MeOH:water:HCl:NH4OH mixture with a molar ratio of $1:2.4:4:10^{-5}:10^{-2}$. The dispersed phase was mixed with the continuous phase (canola oil saturated with ethanol) to form the emulsion (sol:oil phase volume ratio=1:1) and stirred at 1300 rpm and room temperature for 20-30 min. After 24 h aging, the silica gel was filtered to remove the oil phase and then supercritically dried at 318 K, 11.0-12.0 MPa and CO_2 flow of 2-4 $NL \cdot min^{-1}$ during 4h.

[1] García-González, C. A., Alnaief, M., & Smirnova, I. (2011). Polysaccharide-based aerogels - Promising biodegradable carriers for drug delivery systems. *Carbohydrate Polymers, 86*(4), 1425-1438.

[2] García-González, C. A., Carenza, E., Zeng, M., Smirnova, I., & Roig, A. (2012). Design of biocompatible magnetic pectin aerogel monoliths and microspheres. *RSC Advances, 2*(26), 9816-9823.

[3] Alnaief, M., Alzaitoun, M. A., García-González, C. A., & Smirnova, I. (2011). Preparation of biodegradable nanoporous microspherical aerogel based on alginate. *Carbohydrate Polymers, 84*(3), 1011-1018.

[4] García-González, C. A., Uy, J. J., Alnaief, M., & Smirnova, I. (2012). Preparation of tailor-made starch-based aerogel microspheres by the emulsion-gelation method. *Carbohydrate Polymers, 88*(4), 1378-1386.

[5] Alnaief, M., & Smirnova, I. (2011). In situ production of spherical aerogel microparticles. *The Journal of Supercritical Fluids, 55*(3), 1118-1123.
[6] García-González, C. A., Camino-Rey, M. C., Alnaief, M., Zetzl, C., & Smirnova, I. (2012). Supercritical drying of aerogels using CO_2: Effect of extraction time on the end material textural properties. *The Journal of Supercritical Fluids, 66*, 297-306.