

# Supercritical CO<sub>2</sub> Impregnation of AlphaTocopherol in Different Aerogels

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$\alpha$ -tocopherol (TOC), a poorly water-soluble vitamin, was impregnated using supercritical carbon dioxide in two different porous supports: an inorganic one (silica aerogel, SA) and a biopolymer one (maize starch aerogel, MSA). The composite systems can be used for the attainment of novel delivery systems with a rapid or controlled vitamin dissolution rate. TOC impregnation experiments on both the supports were carried out at a pressure of 15 MPa and a temperature of 60 °C. Impregnation equilibrium data were measured and represented as isotherms, whereas impregnation kinetic data were obtained by determining the TOC uptake on the two supports at various times. The TOC/aerogel composites were characterized by FESEM analysis and specific surface area determination. To study the properties of the adsorbed aerogels, as vitamin delivery systems, *in vitro* dissolution tests were performed. The dissolution rates of TOC charged in silica aerogel or maize starch aerogel using phosphate buffered saline solution (PBS) were compared with the one of the unprocessed vitamin.

## 1. Introduction

$\alpha$ -tocopherol (vitamin E, TOC) is a fat-soluble vitamin that, after ingestion, is absorbed through the intestinal tract and reaches the blood via the lymph (Thompson, 1971). Vitamin deficiencies due to fat malabsorption may cause severe clinical consequences and may alter calcium metabolism (Slater et al., 2004). Indeed, TOC deficiency may cause neurological dysfunction and myopathies (Drevon, 1991); in this case, vitamin supplements can be necessary. Moreover, lipophilic vitamins, such as  $\alpha$ -tocopherol, are poorly water-soluble and have a slow dissolution rate (Prosapio et al., 2017). Vitamins bioavailability may be improved through different approaches. Commonly, size-reduction techniques, aimed at obtaining microparticles and nanoparticles are used (Merisko-Liversidge et al., 2003); an alternative way is the charging of the active compound on a biocompatible substrate, which can be a film (Concilio et al., 2015), a membrane (Reverchon et al., 2008) or an aerogel (Smirnova et al., 2003). Among them, aerogels are considered as outstanding matrices, due to the open pore structure and to their large surface area. They are commonly obtained from wet gels using freeze-drying (Jin et al., 2004) or supercritical drying (Cardea et al., 2013). Both organic and inorganic aerogels can be used as biocompatible substrates. Silica aerogels, showing very high porosity (90–99 %) and very high surface areas (400–1000 m<sup>2</sup>/g), and were frequently used as the host matrix for oral delivery systems. Their limitation is related to the fact that they are biocompatible (not toxic for the human body) but not biodegradable (suitable for enzymatic decomposition in the body) (Smirnova et al., 2003).

On the contrary, natural polysaccharides based aerogels are biodegradable and can be used as carriers in nutraceutical and pharmaceutical fields because of their low toxicity, renewability and stability (García-González et al., 2011). In the last years, starch, which is one of the most abundant and low-cost polysaccharides, has been frequently used in the form of aerogel as the carrier for controlled release systems (De Marco and Reverchon, 2017). A promising method to adsorb an active substance into a porous structure is the adsorption driven by supercritical carbon dioxide (scCO<sub>2</sub>) (Smirnova et al., 2004). Supercritical carbon dioxide is frequently used in extraction and fractionation (Subra et al., 1998), micronization (De Marco et al., 2013), nanocomposites structures production (Baldino et al., 2016), liposomes formation (Trucillo et al., 2017), drying (Tsygankov et al., 2018) and adsorption (Mehling et al., 2009). In particular, supercritical adsorption is

based on the following principles: the active compound is dissolved in  $\text{scCO}_2$  and impregnated on the porous aerogel by its exposure to this supercritical solution. After the removal of  $\text{scCO}_2$  through a slow depressurization, an active compound-loaded matrix-free of solvent is obtained. Natural polysaccharides based aerogels were used in a limited number of cases (García-González et al., 2011) as drug carriers; recently, they were proposed as matrices which can adsorb vitamins, through supercritical impregnation, to improve their bioavailability (Pantic et al., 2016). In any case, to the best of our knowledge, literature is focused on impregnation into an inorganic substrate or a polymeric one. The comparison between the impregnation of the same active principle into aerogel of different natures is not commonly performed. Therefore, considering that both inorganic and polymeric substrates have advantages and disadvantages, the aim of this work is to compare the supercritical adsorption of alpha-tocopherol (TOC) on two different supports: maize starch aerogel prepared using supercritical drying and silica aerogel. To find out which of the two matrices is most loaded, adsorption isotherms and kinetics were compared. Finally, with the aim of understanding if the obtained porous structures can be used for vitamin delivery, *in vitro* dissolution tests were performed.

## 2. Materials and methods

### 2.1 Materials

Maize starch 85652 (MS) was purchased from Fluka (Italy), ethanol (EtOH, purity 99.5 %) and  $\alpha$ -tocopherol (TOC, MW: 460.71, purity 96 %) were purchased from Sigma-Aldrich (Italy). Hydrophilic silica aerogel (SA) in the form of monolithic blocks was purchased from Merketech Int. (USA).  $\text{CO}_2$  research grade 4.8 was purchased from Morlando Group (Italy). All these products were used as received. Water was distilled using a laboratory water distiller supplied by ISECO S.p.A. (St. Marcel, AO, Italy).

### 2.2 Apparatuses

Maize starch aerogel (MSA) was prepared in a bench-scale apparatus, that mainly consists of a 500 mL stainless steel cylindrical high-pressure vessel, equipped with a high-pressure pump used to deliver carbon dioxide ( $\text{CO}_2$ ). Pressure in the vessel was measured by a test gauge manometer and regulated by a micrometric valve. The temperature was set by a proportional-integral-derivative (PID) controller connected with electrically controlled thin bands. A second collection vessel located downstream of the micrometric valve, whose pressure was regulated by a backpressure valve, was used to recover the liquid solvent. At the exit of the second vessel, the  $\text{CO}_2$  flow rate and the total quantity of carbon dioxide delivered were measured by a rotameter and a dry test meter, respectively.

Adsorption experiments were performed in an autoclave, consisting of a stainless steel high-pressure cylinder with an internal volume of 100 mL. The carbon dioxide was delivered by a diaphragm piston pump having a maximum working pressure of 30 MPa, whereas an impeller mounted on the top cap and driven by a variable velocity electric motor assured the mixing. The autoclave was heated by two thin band heaters whose thermal control was guaranteed by a PID controller. The temperature inside the cylinder was measured by a K-type thermocouple with an accuracy of  $\pm 0.1$  °C. A digital gauge manometer measured the pressure. At the exit of the autoclave, a rotameter was used to measure the  $\text{CO}_2$  flow rate. Depressurization was obtained through a micrometric valve.

### 2.3 Aerogel preparation

Aerogel preparation was obtained through three steps; the first one consists in the attainment of a hydrogel through gelatinization and retrogradation. The gelatinization step consisted of the preparation of a solution at 15% wt in distilled water, stirring for about 24 h to reach the homogeneity, heating up to 110 °C and pouring into cylindrical molds; the retrogradation step, necessary for the rearrangement of the structure, was obtained by putting the samples in a refrigerator at 4 °C for three days.

The second step was the attainment of an alcogel, gradually replacing the water filling the pores of the gel structure by batch equilibration using a succession of ethanol baths at increasing concentration (30 %, 70 %, 90 % and two times 100 % (v/v)) at room temperature (Glenn and Stern, 1999). The equilibration time for each bath was 24 h.

Then, the alcogels were dried using  $\text{scCO}_2$  to form the aerogels using the following procedure: the vessel where the samples were placed was closed and filled with  $\text{scCO}_2$ . When the operating pressure and temperature were reached (20 MPa and 45 °C), drying was performed for five h, using a  $\text{scCO}_2$  flow rate equal to 1 kg/h. A slow depressurization (20 min) was used to bring back the system at atmospheric pressure and recover the aerogels from the vessel.

## 2.4 Adsorption Experiments

Adsorption experiments were performed using a static method (Smirnova et al., 2003). Briefly, a weighed amount of aerogel (about 0.5 g) was wrapped in filter paper placed on the bottom of the vessel, to avoid its contact with the vitamin in the liquid state. To allow contact with scCO<sub>2</sub>, a weighed amount of the vitamin was placed in a small container opened on the top-mounted axially on the impeller. The autoclave was then closed, heated to the fixed temperature and slowly filled with CO<sub>2</sub>. The pressure and temperature values were fixed at 15 MPa and 60 °C, respectively; they were chosen in agreement with an optimization of the operating conditions previously carried out (De Marco and Reverchon, 2017). After these values were reached, the system was incubated for 24 h, which assured the dissolution of the vitamin in scCO<sub>2</sub> and the attainment of the adsorption equilibrium. The amount of carbon dioxide in the vessel was determined from the density value (given at the test temperature and pressure). In particular, at 15 MPa and 60 °C, it was equal to 60.2 g. Then, CO<sub>2</sub> was vented out at a constant flow rate (about 1 MPa/min). When temperature and pressure in the vessel were equal to the ambient, the aerogel was removed from the autoclave and weighed. The weight increase of the aerogel indicated the amount of loaded vitamin, later verified using UV/vis spectrophotometry.

The thermodynamics of adsorption was quantified using adsorption isotherms, relating the concentration of the adsorbate in the scCO<sub>2</sub> phase to the concentration of the adsorbate in the solid phase. Adsorption isotherms were measured at 60 °C/15 MPa for both the substrates. The kinetics of adsorption was linked with the time the vitamin took to reach the equilibrium concentration, under given conditions of temperature and pressure. Kinetic data were obtained by determining, for both the substrates, the loading within the aerogel at various times from 1 to 24 h at 15 MPa and 60 °C.

## 2.5 Analytical Methods

Samples of the loaded aerogels were characterized by specific surface area determination and in vitro dissolution tests. The surface area of the aerogels was characterized by ultra-high-purity nitrogen adsorption and desorption isotherms at -176 °C using the Brunauer, Emmet and Teller (BET) equation (Quantachrome Instruments, mod. Nova 1200e, Kingsville, TX). Before the measurement, about 200 mg of the sample was heated at 110 °C for two h under vacuum. For the determination of the surface area, adsorption isotherms in the linear region of the BET plot (at a relative pressure  $p/p_0$  in the range 0.05–0.3) using a multipoint BET were determined. Dissolution studies were performed using a UV/vis spectrophotometer (model Cary 50, Varian, Palo Alto, CA). The release medium was constituted by a 0.1M sodium dodecyl sulfate (SDS)/0.1M sodium chloride (NaCl) solution. Accurately weighted samples containing an equivalent amount of tocopherol (1 mg) were suspended in 4 mL of medium and placed into a dialysis sac; it was then incubated in 500 mL of medium, continuously stirred at 200 rpm and 37 °C. Each analysis was carried out in triplicate and the proposed curves are the mean profiles. Vitamin loading was measured by UV–vis analysis, measuring the absorbance ( $\lambda=292$  nm) obtained in the release medium at the end of the vitamin release, i.e. when all the vitamin was released from the aerogel to the outer water phase. The absorbance, then, was converted into vitamin concentration using a calibration curve.

## 3. Results and discussion

The experimental work can be divided into the following steps, corresponding to three subsections:

- study of adsorption isotherms to know the relationship between the concentration of TOC in the fluid phase and the solid phase at equilibrium at the chosen temperature and pressure;
- study of adsorption kinetics of TOC in MSA and SA, to calculate the time necessary to reach equilibrium conditions;
- characterization of the adsorbed aerogels.

### 3.1 Adsorption isotherms

The adsorption isotherms of  $\alpha$ -tocopherol on MSA and SA were obtained at 60 °C/15 MPa. To ensure that equilibrium was reached, the adsorption process was carried out for a period of 24 h. The obtained isotherms are presented in Figure 1. The concentration of the vitamin on both the aerogels increases with its concentration in CO<sub>2</sub>; the maximum loading obtained is 0.585 mmol/g, in the case of silica aerogel, and 0.529 mmol/g, in the case of maize starch aerogel. Therefore, slightly larger adsorption is obtained considering the silica aerogel as support.

### 3.2 Kinetics of TOC adsorption into silica and starch aerogel

To evaluate the time needed for the complete adsorption of the  $\alpha$ -tocopherol on the two supports, the adsorption kinetics were determined. Uptake was expressed as loading %, grams of vitamin per gram of aerogel.

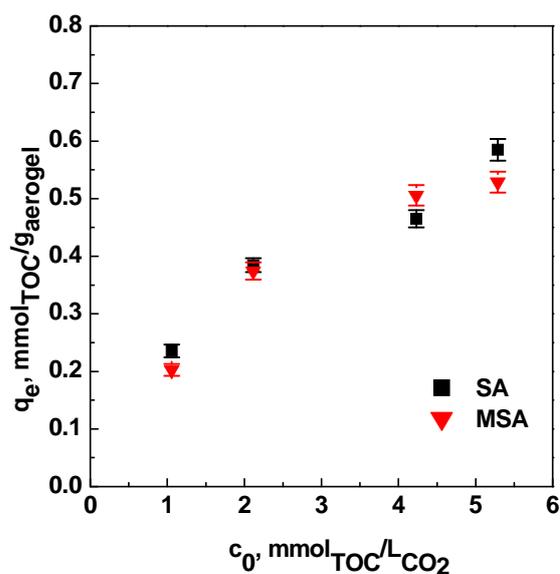


Figure 1: Adsorption isotherms of tocopherol on silica aerogel and maize starch aerogel

These data were obtained charging a weighed quantity of the vitamin, corresponding to the saturation concentration in CO<sub>2</sub> at the given pressure and temperature, dissolving it in scCO<sub>2</sub>, and contacting it with the aerogel for various time intervals (from 1 to 48 h). In Figure 2, the adsorption kinetics obtained at 15 MPa and 60 °C are reported for both the supports.

Data in Figure 2 suggest that for both the supports, the speed in reaching the equilibrium value is similar: indeed, both for MSA and SA, 90 % of the maximum quantity of  $\alpha$ -tocopherol was adsorbed in 6 h. The maximum uptake was obtained considering SA as support. This result is explainable because it is known from literature data that the surface area of silica aerogel is higher than the one of organic aerogels.

### 3.3 Characterization of aerogels after adsorption

In vitro dissolution tests were performed using UV-vis spectroscopy, to determine the effective quantity of vitamin in the aerogels and the modification of its dissolution rate. The analyses were performed on MSA/TOC and SA/TOC composites; these analyses revealed that the samples of MSA/TOC and SA/TOC showed a vitamin content ranging between 95 % and 98 % of the expected loaded values.

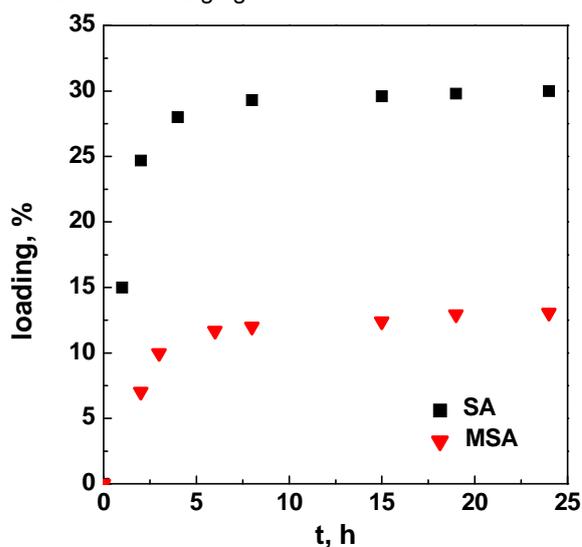


Figure 2: Adsorption kinetics of tocopherol on silica aerogel and maize starch aerogel

In the dissolution tests, the dissolution profile of the unprocessed vitamin was compared with physical mixture MSA/TOC and SA/TOC and with MSA and SA loaded with vitamin; the dissolution rate of each sample in the medium was monitored, plotting the percentage of dissolved vitamin as a function of time. In Figure 3, all the dissolution profiles are reported. The difference between the triplicates was less than 1% and, therefore, the error bars are not visible. It is possible to observe that unprocessed TOC and both the physical mixtures MSA/TOC and SA/TOC achieve the complete dissolution in about 1200 min corresponding to 20 h; whereas, TOC adsorbed onto MSA arrives at 100 % in about 75 min, and TOC adsorbed onto SA needs about 5000 min, corresponding to 83 h to reach the complete release.

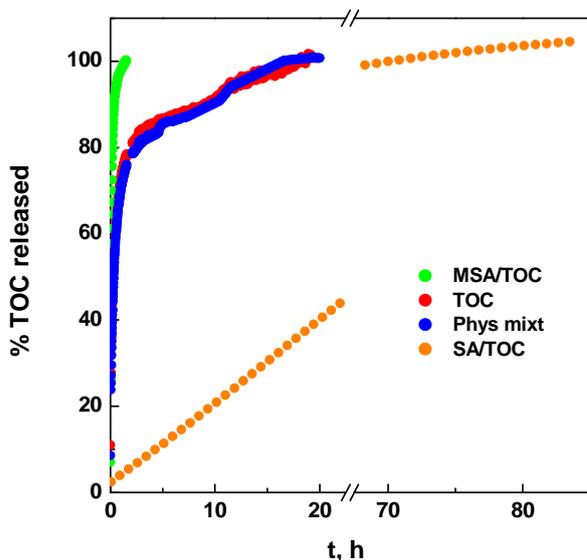


Figure 3: Release profiles of unprocessed TOC, physical mixtures and TOC adsorbed in MSA and SA

Therefore, MSA and SA can be used as TOC supports for different applications. Indeed, the use of maize starch aerogel as carrier promotes a fast release of the vitamin, whereas silica aerogel can be used for a delayed vitamin release.

The surface areas of MSA and SA before and after exposure to  $scCO_2$  was measured through nitrogen adsorption using the BET adsorption isotherms. The surface areas do not change significantly during the process. Indeed, the surface area of the untreated aerogel is equal to  $90\text{ m}^2/\text{g}$  in the case of MSA, whereas it is equal to  $800\text{ m}^2/\text{g}$  in the case of silica aerogel. After the adsorption with TOC, the surface areas are equal to  $86$  and  $770\text{ m}^2/\text{g}$  in the case of TOC loaded onto MSA and SA, respectively. Therefore, they are reduced by about 4 % compared to untreated MSA and SA.

#### 4. Conclusions

In this work, it was demonstrated that the supercritical fluid adsorption could be considered an effective way to incorporate poorly water-soluble vitamins (vitamin E in this case) into different aerogels, such as maize starch aerogel and silica aerogel. The best results, in terms of loading, were obtained using the silica aerogel, considering that, in this case, the maximum amount of loaded vitamin is more than double that the one onto MSA. Indeed, in the case of SA/TOC composites, a loading equal to 30 % was obtained, whereas, in the case of MSA/TOC, a loading equal to 13 % was achieved.

Dissolution tests confirmed the strong modification of the dissolution rate of the loaded aerogel compared to the unprocessed vitamin; indeed, it is 16 times faster than the unprocessed vitamin, in the case of maize starch aerogel and 4 times slower than the unprocessed  $\alpha$ -tocopherol in the case of silica aerogel.

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