

A New Tool to Generate PVDF-HFP + Curcumin Biocomposite by Supercritical Assisted Gel Drying

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In this work Poly(vinylidene fluoride-co-hexafluoropropylene) (PVDF-HFP) aerogels loaded with curcumin were produced by a supercritical CO₂ assisted process. The aim was to create controlled release systems for wound healing applications. Performing the supercritical gel drying at suitable operating conditions (200 bar and 45°C), PVDF-HFP aerogels were generated starting from gels at 5, 10 and 12% w/w PVDF-HFP. They presented interesting morphologies: nanoporous, homogeneous and regular. Then, PVDF-HFP gels were loaded at 3% w/w of curcumin with respect to the polymer and, subsequently, dried by SC-CO₂ at the same operative conditions previously tested. Curcumin release tests and DPPH tests were performed on the composite systems, for the determination of the curcumin antioxidant activity. The release test showed that the PVDF-HFP aerogels allowed to obtain a regular and prolonged release of curcumin, up to 45 h; furthermore, DPPH tests confirmed that curcumin still presented a high antioxidant activity (about 77%). All the samples produced were also characterized from a chemical and physical point of view: from the results of DSC and XRD analyses, it was observed that the gel drying process assisted by SC-CO₂ did not lead to any change in the properties of the polymer (PVDF-HFP) and of the active agent (curcumin).

1. Introduction

Aerogels are widely used in the commercial, technical and scientific sectors thanks to their remarkable properties (Hrubesch, 1998): high specific surface area, homogeneity, elasticity, insulating solid, low refractive, low dielectric constant. They can be obtained using drying technologies; but, traditional procedures, such as air drying and freeze drying, are not able to maintain the delicate structure of the gel and can generate xerogels (Valentin et al., 2005). Indeed, these methods involve the formation of liquid mixtures in the nanoporous gels, and, with the removal of the solvent, the surface tension of the liquid exerts a capillary pressure on the pore wall which causes the compression of the structure and its consequent collapse.

Carbon dioxide (CO₂) is one of the ideal supercritical fluids used for a variety of processes, because of its peculiar characteristics (Marra et al., 2012; Baldino et al., 2017a; Baldino et al., 2017b; Sarno et al., 2016). Indeed, it is used for micronization (De Marco et al., 2015b), coprecipitation (Prosapio et al., 2016), electrospray and electrospinning (Baldino et al., 2019), scaffolds (Cardea et al., 2014), membranes formation (Reverchon et al., 2007), and gel drying (Baldino et al., 2016b). In particular, CO₂ at supercritical conditions, thanks to its mild critical conditions, is suitable for the treatment of numerous polymers (Reverchon et al., 2008). There are several examples of polymeric aerogels successfully obtained by supercritical drying process (De Marco et al., 2015a). Martins et al. (2015) produced alginate aerogels cross-linked with calcium starch characterized by both meso- and microporosity. In particular, aerogels are a very interesting class of materials in the biomedical field, due to their porous structure and high surface area; however, they are often characterized only by mesoporous structures. The authors succeeded in obtaining both types of porosity by carrying out a multi-step process: at the beginning the formation of hydrogels in an aqueous medium and the cross-linking of the calcium starch-alginate mixture by treatment with CO₂ in an autoclave was performed; then, in order to introduce macroporosity, the autoclave was depressurized with different speeds (0.1, 10, 30 bar/min). Finally, these hydrogels were exchanged in ethanol and the alcogels obtained were dried using a supercritical drying process, conducted for 4 h at 120 bar and 40°C. Morphological analysis revealed a high interconnectivity and a pore size greater than 100 microns. The presence of both types of porosity can lead

advantages in terms of cells adhesion (due to nanoporosity) and growth (due to microporosity that facilitates nutrients diffusion). Furthermore, there was a significant increase in macroporosity from 2 to 25% as the CO₂ depressurization rate increased. Garcia-Gonzales et al. (2011) produced maize starch aerogels, in the form of microspheres, through a process that first involved the creation of an emulsion with vegetable oil, then, the gel phase and, finally, the supercritical drying, without the use of chemical crosslinkers. The intrinsic biocompatibility and biodegradability of starch allowed its use as a chemical carrier in many applications. The main parameter that influenced the structural properties of these aerogels was the gelation temperature: a specific surface area of 112 m²/g was measured using BET analysis, at a gelation temperature of 393 K; whereas, at a temperature of 413 K, there was a decrease in the specific surface area, which was 40 m²/g. By increasing the ratio of vegetable oil/starch to aqueous solution, an improvement in the sphericity of the particles and a reduction in their size was observed. Finally, the load capacity of these particles was measured, using ketoprofen as the model compound, and a maximum load capacity of 16% w/w was obtained. Baldino et al. (2015) produced alginate-gelatin and chitosan-gelatin aerogels by supercritical gel drying. The alginate-gelatin aerogels were obtained starting from aqueous solutions having 5% w/w of each biopolymer and an aqueous solution of 5% w/w calcium chloride was used for cross-linking. The hydrogels obtained were subjected to substitution baths using ethanol and, then, processed by supercritical drying at 200 bar and 35°C. The chitosan-gelatin aerogels, on the other hand, were prepared with solutions of acetic acid at pH= 2.45 and at 5% w/w of each biopolymer; an aqueous solution of 1% w/w glutaraldehyde was used for cross-linking. Also in this case, water was replaced with ethanol, before the drying process. In both cases, the uniform nanostructure characterizing the starting hydrogels remained intact downstream of drying assisted by SC-CO₂. In fact, during the process, a mixture of CO₂ and organic solvent having a negligible surface tension was generated, which allowed to preserve the morphology of the samples. All the aerogels produced were characterized by porosity values above 80%.

The interesting peculiarities of the generated aerogels, attracted numerous researcher in the study of this materials as drug controlled release devices (Garcia-Gonzales et al., 2011). In particular, in this work, we focused our attention on the production of polyvinylidene fluoride-hexafluoropropylene (PVDF-HFP) aerogels loaded with curcumin, for biomedical applications (i.e., biocomposites for wound healing applications) by supercritical drying process.

PVDF is a fluoropolymer (i.e., a polymer containing fluorine atoms), extremely pure and generally used for the production of porous structures (Cardea et al., 2009). It has many features, such as high hydrophobicity, low density, low melting point and high resistance to acids, bases, solvents and heat (Mishra et al., 2014); moreover, it has been already successfully processed by supercritical drying for the formation of nanoporous aerogels (Cardea et al., 2009). Curcumin is used in traditional medicine to treat disorders such as anorexia, cough, sinusitis and liver disease (Eignera et al., 1999). Other studies in vivo and in vitro models revealed that curcumin has anti-bacterial, anti-oxidant, anti-inflammatory and anti-carcinogenic properties. Furthermore, curcumin is associated with antiviral and wound healing and, being a hydrophobic polyphenol, it is insoluble in water, but it is soluble in many inorganic solvents, such as ethanol and acetone (Aggarwal et al., 2007).

2. Materials and methods

The loaded aerogels were obtained using the following materials bought from Sigma Aldrich (USA): PVDF-HFP with average molecular weight Mn= 130,000, curcumin with purity> 65%, acetone with degree of purity> 99.5% and ethanol with degree of purity> 99.9%. CO₂ (purity 99.9%) was bought by Morlando Group S.R.L. (Sant'Antimo, NA, Italy).

2.1 PVDF-HFP/curcumin biocomposites production by supercritical drying

For the formation of the loaded gel, acetone was used as solvent (with a fixed mass fraction) and ethanol as non-solvent for PVDF-HFP. Solutions were obtained at 5, 10 and 12% w/w PVDF-HFP in acetone (60% w/w) and adding 3% w/w of curcumin with respect to PVDF-HFP. Gels were prepared by mixing at T= 50°C the solutions of PVDF-HFP+curcumin and acetone to which ethanol was subsequently added dropwise. The polymer/drug solutions were mixed until completely homogenization and, then, placed in steel cylinders (with a diameter of about 2 cm) and subjected to a freezing phase for about 2 h at -20°C, in order to obtain the formation of gels. The supercritical drying was performed in a home-made apparatus already described in previous works (Baldino et al., 2016a) at the operating conditions of 200 bar and 45°C, for 4 h. At these conditions, when the supercritical CO₂ (SC-CO₂) diffused into the gel, formed a supercritical mixture with the liquids it contained (acetone and ethanol). This mixture was characterized by a surface tension close to zero. Therefore, given the absence of cohesive forces between the fluid and the solid phase, SC-CO₂ was able to effectively remove the organic solvents from the nanostructured polymeric network, avoiding its structural collapse (Baldino et al., 2016b).

2.2 Biocomposite characterizations

PVDF-HFP aerogels loaded with curcumin were cryofractured with liquid nitrogen and subsequently coated with gold at 30 mA for 150 s (Auto Sputter Coater mod. 108 A, Stansted, UK). Samples were morphologically characterized with a Scanning Electronic Microscope (SEM mod. LEO420, Assing, Italy).

DSC analysis was performed on pure PVDF-HFP, pure curcumin and on the loaded aerogels (PVDF-HFP + curcumin) by a DSC 30 Mettler Toledo.

XRD measurements were performed with a Bruker D8 X-ray diffractometer using CuK α radiation.

Curcumin release tests from PVDF-HFP loaded structures were performed using a Varian (mod. Cary 50) UV/Vis spectrophotometer and measuring the absorbance at 430 nm; i.e., the wavelength of curcumin maximum absorption. The samples were immersed in 25 mL of a phosphate buffer solution at pH of 4.5 and at temperature of 25°C.

The antioxidant activity of the biocomposite PVDF-HFP + curcumin was measured by the DPPH method. A 0.1 mM solution of DPPH was prepared in ethanol and 1 mL of this solution was added to 3 mL of curcumin in phosphate buffer solution, recovered from the release tests performed before. This final solution was mixed by a Vortex for 2 min and incubated for 30 min at room temperature (in the dark). The absorbance was measured at 517 nm. The antioxidant activity of curcumin was calculated using the equation (1):

$$I(\%) = (1 - A_s/A_c) \times 100 \quad (1)$$

where A_c is the absorbance of the control (1 mL, DPPH solution without curcumin) and A_s is the absorbance of the solution with curcumin (Ak et al., 2008).

3. Results and discussion

In the first part of the work, the morphology of the loaded aerogels was observed. In figure 1, SEM images of PVDF-HFP + curcumin biocomposite structures obtained at different concentrations of PVDF-HFP, are reported.

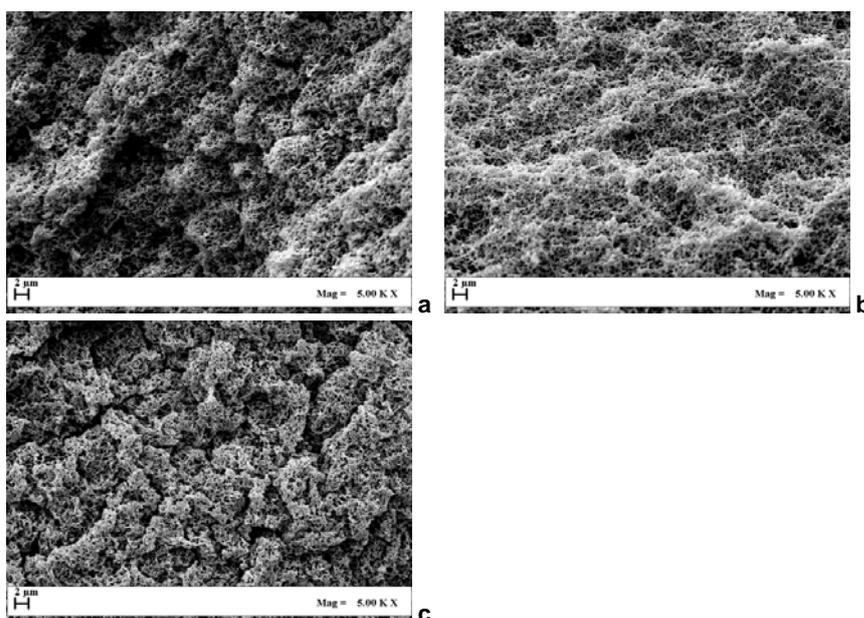


Figure 1: SEM images of PVDF-HFP aerogels loaded with 3% w/w of curcumin obtained at different PVDF-HFP concentrations: 5% w/w a), 10% w/w b), 12% w/w c).

From SEM analysis of the PVDF-HFP aerogels at 5, 10 and 12% w/w PVDF-HFP loaded with 3% w/w curcumin with respect to the polymer, obtained by supercritical drying at 200 bar and 45°C, it was possible to observe a nanoporous and homogeneous section morphology for all the samples, as in the case of pure PVDF-HFP aerogels (Cardea et al., 2009). Therefore, the presence of curcumin did not influence the formation of aerogels during the process assisted by SC-CO₂, and, thanks to the uniform and symmetrical nanostructured morphology, they are also suitable for the creation of controlled release systems of an active principle.

Subsequently, DSC analyses were performed on pure PVDF-HFP, on pure curcumin and on one of the loaded aerogel generated (i.e., 10% w/w PVDF-HFP aerogel + 3% w/w curcumin). In figure 2, the DSC traces of the analyzed samples are reported.

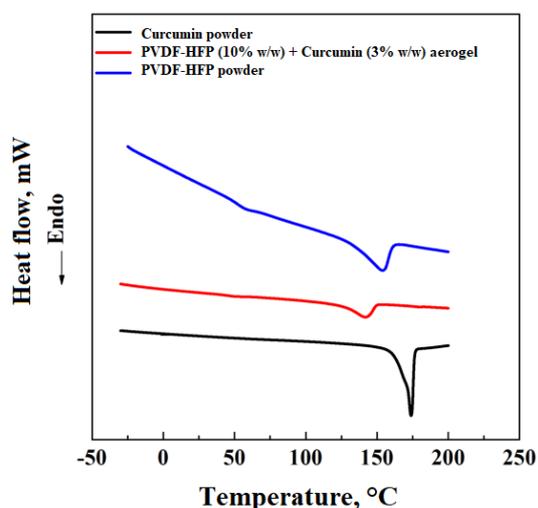


Figure 2: DSC analyses of pure PVDF-HFP, pure curcumin and 10% w/w PVDF-HFP aerogel loaded with 3% w/w curcumin.

DSC analysis showed that the polymer (PVDF-HFP) had a melting peak (T_m) around 155°C and that curcumin had a melting peak (T_m) around 175°C. These peaks were also preserved downstream of the supercritical process conducted to obtain the aerogels. This means that the process has not altered neither the properties of the polymer, which remained semi-crystalline, nor of curcumin, which remained crystalline even when it was loaded into the polymeric matrices. Probably this was favored by the use of a semi-crystalline polymer, such as PVDF-HFP, which could act as a stabilizing agent for curcumin crystals, preventing their amorphization.

The results obtained from the DSC analysis were also confirmed by the diffraction spectra obtained by XRD analysis. In the diffraction spectra of the aerogels loaded with curcumin, and processed by SC-CO₂ drying, both the peaks representing the crystalline phases of the polymer and the peaks representing the crystalline phases of the curcumin were present.

In the last part of this work, the loaded aerogels were subjected to the release test. The aim was to determine their applicability as controlled release systems, once the kinetics of curcumin release from the polymeric matrix was measured in dependence of the polymer percentage and of the aerogel morphology.

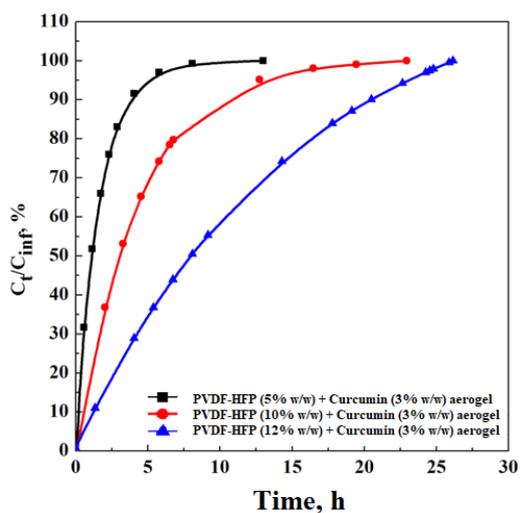


Figure 3: Curcumin release curves from aerogels at 5, 10 and 12% w/w PVDF-HFP, obtained at 200 bar and 45°C.

From figure 3, it was observed that the quantity of curcumin released in the medium simulating the physiological conditions, increased with time, until an equilibrium value was reached. The trend of these curves was asymptotic exponential. Furthermore, from the release profile analysis, it appeared that the curcumin release rate was inversely proportional to the polymer concentration within the aerogels. In particular, it is possible to notice that the increase of the PVDF-HFP content increased the time necessary to reach the maximum quantity of curcumin released, because the release rate of the active agent decreased due to the mass transfer resistance. Observing the times for reaching the maximum curcumin value in the medium, it can be seen that in the case of 12% w/w PVDF-HFP aerogel, the larger amount of polymer, determined an increase in the mass transfer resistance and, consequently, slowed down the release process of the active agent (i.e., curcumin) encapsulated therein. Therefore, the aerogel morphology significantly affected the release profiles of the active agent. Comparing the release times, it is possible to notice that, as the polymer concentration increased, the diffusion rate of the simulating medium the physiological conditions in aerogel decreased. Aerogels with a larger polymer percentage were characterized by a more compact and homogeneous structure with pores of smaller diameter; this involved an increase in the mass transfer resistance and, therefore, a slow and gradual release over time of the active agent. Summarizing, as the polymer concentration increased, the release rate of curcumin decreased and, consequently, the release was prolonged respectively for 12, 20 and 26 h, for aerogel at 5, 10 and 12% w/w PVDF-HFP.

The antioxidant activity of curcumin loaded into the PVDF-HFP polymeric aerogel was analyzed in terms of anti-radical activity, using the DPPH method. The antioxidant activity was calculated taking into account the decrease in absorbance, observed following radical capture; more precisely, as a percentage of inhibition of DPPH radical formation according to the equation reported before (1). The results are shown in table 1.

Table 1: Antioxidant activity expressed as a percentage of inhibition of DPPH radical formation.

Aerogel	I (%)
5% w/w PVDF-HFP + 3% Curcumin	35.93
10% w/w PVDF-HFP + 3% Curcumin	55.86
12% w/w PVDF-HFP + 3% Curcumin	76.83

As expected, the antioxidant activity was a function of the concentration of curcumin released in the simulant; in particular, it increased as the amount of curcumin loaded increased, since it was the 3% w/w with respect to the polymer. All the aerogels showed a fairly high antioxidant activity, also considering the small percentage of curcumin that was used. A maximum antioxidant activity of curcumin of about 77% was reached for PVDF-HFP aerogels at 12% w/w, due to the larger content of curcumin.

4. Conclusions

In this work, polymeric PVDF-HFP aerogels loaded with an active agent, curcumin, were produced using supercritical drying process, to determine the possibility of using them as controlled release systems and, in particular, as patches to promote wound healing. Aerogels at 5, 10 and 12% w/w PVDF-HFP showed a nanoporous, symmetric and uniform morphology and, therefore, they were suitable for the creation of controlled-release polymeric systems. These aerogels, loaded at 3% w/w curcumin with respect to the polymer, were subjected to release tests. It has been observed that the release time of the active agent increased as the percentage of polymer used increased; in particular, the maximum curcumin concentration was reached in 12, 20 and 26 h, respectively, for the aerogels at 5, 10 and 12% w/w of polymer. Furthermore, since curcumin is a strong antioxidant agent, its antiradical activity was also measured, reaching a maximum inhibition percentage of about 77% for the PVDF-HFP aerogel at 12% w/w.

For the completion of the study the specific surface of the aerogels will be evaluated by BET. Moreover, it could be tried to further optimize the release time of the active agent and to increase the quantity of curcumin loaded inside the aerogels. Finally, in vivo studies should be performed to assess the effectiveness of these systems as "devices" that favor wound healing processes.

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