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3-D loaded scaffolds obtained by supercritical CO₂ assisted process

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Abstract. In this work, a supercritical CO₂ (SC-CO₂) drying process for the formation of 3-D PVDF-HFP loaded scaffolds was tested. Experiments at pressures ranging between 150 and 250 bar and at temperatures ranging between 35 and 55 °C were performed. The PVDF-HFP-acetone-ethanol solution at 15% w/w polymer was selected as the base case. The drug (amoxicillin) concentration was varied from 20 to 30% w/w with respect to PVDF-HFP. SC-CO₂ drying process was confirmed to be a valid alternative to generate loaded structures; indeed, scaffolds characterized by nanometric networks (with mean pore diameter of about 300 nm) with a homogeneous drug distribution were obtained. Drug controlled release experiments were also performed and a quasi-zero order release kinetic was observed.

1. Introduction

Poly(vinylidene fluoride) PVDF is frequently copolymerized with hexafluoropropylene (HFP), in order to obtain the PVDF-HFP, which is a material with a good resistance to acid environments, semi-crystalline and, furthermore, biocompatible. For these particular characteristics, various applications have been tested for PVDF-HFP such as electrical [1], catalytic [2] and biomedical [3-4] applications. Among biomedical applications, one of the most interesting is the use of loaded scaffolds, with one or more active principles, as controlled release devices; the aim is to control the release of a drug in a way that its administration to the patient is delayed or prolonged in time. Indeed, during the administration of a drug, haematic concentration peaks usually have to be avoided; however, with traditional pharmaceutical devices, such as tablets, this problem is very serious and worrying, and difficult to be solved [5]. Different mass transfer mechanisms are involved in the release of an active principle from a controlled release system, depending on the characteristics of the materials used and on the scaffolds morphology. In particular, the mechanisms of swelling, erosion and/or diffusion have to be considered in order to deeply study the phenomena. Regarding the characteristic of PVDF-HFP (non-swelling and non-degradable material), the use of scaffolds of this polymer in drug release processes implies the consideration of diffusivity mechanisms.

In the last 20 years, various processes have been tested for the generation of loaded scaffolds for controlled release applications such as phase separation [6-7], foaming combined with particulate leaching [8], solvent casting [9], gel drying [10-11], etc. This last process is probably the most interesting because by means of it, it is possible to assure the formation of homogenous and skinless nanostructured porous scaffolds. In any case, all the processes indicated above present several



problems; for example, long processing time (12-48 h) that can imply the stratification of the drug inside the scaffolds due to the separation of the loaded materials from the polymeric solutions. In this way, the efficiency of the generated devices sensibly decreases due to the inhomogeneous distribution of the drug. Moreover, traditional processes tested until now do not allow the complete removal of the organic solvents involved in the starting solutions, and the presence of solvent residue can compromise the efficiency of the controlled release device, too.

Considering the gel drying process, it is important to eliminate the solvent contained in the gel. Indeed, during gel drying, the polymeric structure can collapse due to the liquid surface tension; this effect is related to the action of cohesive forces that act between the liquid and the nanosized network of the polymer. In a previous work, our research group used a supercritical carbon dioxide (SC-CO₂) drying technique to generate PVDF-HFP aerogels with significant porosities [12]. This process can allow some benefits with respect to the traditional processes, thanks to the very interesting peculiarities of the CO₂ at the supercritical conditions, such as high diffusivity, low viscosity and high affinity to several organic solvents [13-14]. In particular, PVDF-HFP nanoporous aerogel was obtained without residual solvents and in very short processing times.

The target presented in this article is to test, for the first time, the SC-CO₂ drying technique to generate PVDF-HFP drug loaded scaffolds for controlled release applications. The morphology of the scaffolds and the behavior of the drug (i.e., amoxicillin, an antibiotic) in the system generated will be studied.

2. Materials and Methods

2.1 Materials

PVDF-HFP (density 1.78 g/cm³) was kindly provided by Solvay S.A. (Ixelles, Belgium); amoxicillin (purity 99.6%), acetone (purity 99.8%) and ethanol (purity 99.7%) were purchased from Sigma-Aldrich; carbon dioxide (purity 99.5%) was bought from Alfa Ossigeno (Fisciano, Italy).

2.2 Generation of loaded scaffolds

To prepare the scaffolds, PVDF-HFP was dissolved in acetone at a concentration of 15% w/w. The solution was stirred and warmed at 55 °C, in order to obtain a homogeneous solution, to which different amounts of amoxicillin were added; a drug suspension (amoxicillin is insoluble in acetone) was then obtained. Then, the non-solvent (ethanol) was added. The obtained suspensions were placed in proper formation cells and put at -30 °C in a deep-freezer. The residence time was properly chosen in order to obtain the gel formation.

The bench apparatus used for the SC-drying process was mainly constituted by a 316 stainless steel high-pressure vessel (I.V. = 80 mL). Once the gel was charged, the vessel was closed. Then, a high-pressure pump (Lewa, Germany) was used to produce the desired pressure, by pumping CO₂ from the bottom of the vessel. The CO₂ flux (at a constant flow rate of 1.5 kg/h) was assured in continuous mode for about 1.5 h. Then, the vessel was rapidly depressurized (at 25 bar/ min) and, once atmospheric pressure was reached, the scaffold was removed from the support. The experiments were performed at pressures ranging between 150 and 250 bar and temperatures ranging between 35 and 55 °C.

2.3 Scanning electron microscopy (SEM) and Energy Dispersive X-Ray analysis (EDX).

The generated scaffolds were cut with a microtome (Bio-optica S.p.A, Italy, Mod. Microm HM 550 OMVP); a sample was sputter coated with gold and observed with scanning electron microscope (SEM - mod. LEO 420, Assing, Italy), in order to study the morphology and drug distribution. An image analysis software program (Sigma Scan Pro 5.0, Jandel scientific, San Rafael, Canada) and Origin 7 (Microcal, Northampton, USA) were used to measure the mean pore diameter.

To determine the amoxicillin dispersion in the scaffold, the samples were coated with chromium (layer thickness 150 Å). Then, an Energy Dispersive X-Ray (EDX) analyzer was used to detect the signal of Sulphur atoms (present in amoxicillin crystals, but not in the polymer).

2.4 Drug release analysis

Drug release tests were performed to study the kinetics of amoxicillin release from PVDF-HFP scaffolds. PVDF-HFP scaffolds were immersed in a glass flask with 1 liter of a physiological saline solution, at pH 7.2, used as drug release medium. The flask was sealed and put in a heater set at 37 °C and with a stir of 250 rpm. Every 10 minutes, the amoxicillin concentration was measured using an ultraviolet spectrophotometer.

3. Results and Discussion

3.1 Effect of supercritical fluid

The use of supercritical fluids is very important in the generation of aerogels; indeed, one of the main problems of gel drying is related to the surface tension of the liquid solvent that has to be removed, which can determine solid structure collapse. Considering that a fluid at supercritical conditions has no surface tension, the use of SC-CO₂ can allow the complete elimination of the organic solvents from the starting gel. However, it could also be insufficient to adequately process the gel; indeed, when SC-CO₂ diffuses into gel, it forms a supercritical mixture with the solvents used during the preparation of the gel, in this case acetone and ethanol. When the mixture SC-CO₂-acetone-ethanol is at supercritical conditions too, very low surface tension conditions are reached. Analyzing the temperatures used during the experimentation (ranging between 35 and 55 °C), the mixture critical point (MCP) of both systems (i.e. CO₂-ethanol and CO₂-acetone) is at pressures lower than 100 bar [15-16]; considering that pressures higher than 150 bar (i.e. from 150 to 250 bar) were used, supercritical conditions were obtained in each operative condition.

Then, the gel structure is not destroyed and the scaffold generated by SC-CO₂ drying process conserves sizes and tridimensional (3-D) form of the starting gel. Moreover, the high affinity of the SC-CO₂ with the solvents used allowed complete elimination of them. The result was the formation of 3-D stable and dry devices that can work as scaffold for tissue engineering applications. Once the feasibility of the SC-CO₂ drying technique to obtain 3-D scaffolds was verified, we focused our attention on the generation of loaded scaffolds.

3.2 Effect of drug concentration

A solution containing PVDF-HFP at 15% w/w, acetone at 60% w/w and ethanol at 25% w/w was processed, and added different quantities of drug (i.e. 20 and 30% w/w of polymer). SEM and EDX images, presented in Figure 1, report PVDF-HPF loaded scaffold generated at 250 bar and 35 °C with 20% w/w of amoxicillin loaded inside. As seen in Figures 1a and 1b, the formation of a homogenous nanoporous structure is evident, very similar to those obtained for unloaded PVDF-HFP structures [12]; this result is very important because it confirms that the loading of an active principle in the starting solution did not condition the formation of the gel and the supercritical process efficiency. The porosity of the structure generated was very high (> 85%) and no residual solvents were present (lower than 5 ppm). Moreover, porous skins and pores with average diameter of about 300 nm were obtained.

Analyzing Figure 1c, in which EDX is reported, it is possible to observe the location of the drug crystals inside the scaffold; indeed, the sulfur atoms, which are the characteristic element of amoxicillin, are indicated with the green color. As a consequence, a very good distribution of the drug along the scaffold section can be put in evidence. This result is very important and interesting because, as reported in the introduction section, one of the main problems of the traditional loading techniques

concerns the stratification of the drug along the structure, and the consequent low efficiency of the generated devices (possible burst effect phenomena).

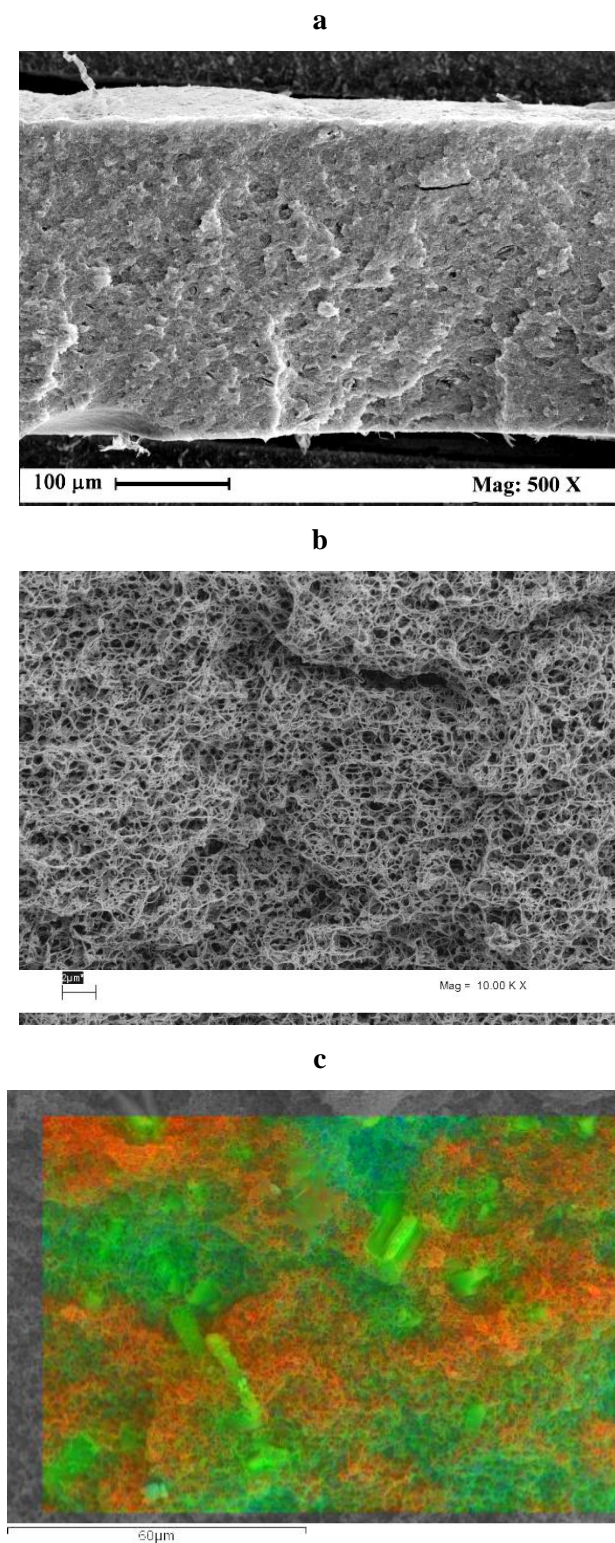


Figure 1 SEM and EDX analyses of scaffolds generated at 250 bar and 35 °C with 20% w/w of amoxicillin: (a) scaffold section, (b) higher enlargement of scaffold section, (c) EDX.

3.3 Effect of SC-CO₂ solvent power

Experiments were performed at different pressures (150, 200 and 250 bar) and temperatures (35, 45 and 55 °C) maintaining the polymer concentration at 15% w/w and drug concentration at 20% w/w. It was observed that the modification of the process parameters did not affect the scaffolds' final morphology and characteristics. It is known that changing the operative conditions lead to the changes of the characteristics of the SC-CO₂, such as solvent power, viscosity and diffusivity. As indicated before, these characteristics are very important for the success of the supercritical drying procedure, but they do not interfere with the gel formation (that occurs in the freezer, before the drying step). As a consequence, the supercritical drying procedure can be considered as an on/off technique that can (or cannot) give efficient results in terms of gel drying, complete elimination of the solvents and preserving of the nanoporous network of the gel, but is not able to modify the aerogel final morphology.

3.4 Drug release analysis

In Figure 2, release curves of pure amoxicillin (· · ·), PVDF-HFP scaffolds with 20% w/w of amoxicillin (—), PVDF-HFP scaffolds with 30% w/w of amoxicillin (- - -), generated at 250 bar and 35 °C, are presented.

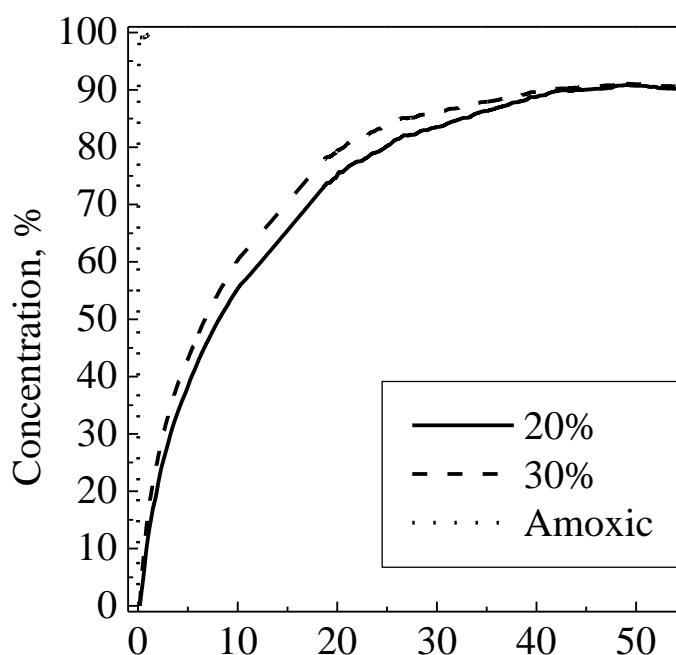


Figure 2 Release curves of pure amoxicillin (· · ·), PVDF-HFP scaffolds with 20% w/w of amoxicillin (—), PVDF-HFP scaffolds with 30% w/w of amoxicillin (- - -), generated at 250 bar and 35 °C

From the diagram presented, it can be observed that the drug was completely solubilized in 10 minutes, validating the idea that, in this case, a control of the release is necessary. From the release curves of the PVDF-HFP scaffolds, it is evident that the time of drug release increased to 50 h for both scaffolds (i.e. with 20% and 30% of drug). The concentration of amoxicillin in the starting solutions had no effect on the qualitative and quantitative trends of the release curves.

Therefore, the scaffolds morphology allowed control of the drug release. This result is related to the nanoporous structure of the PVDF-HFP scaffolds, which involves slow diffusions of the physiological solution into the scaffolds and of the physiological solution + drug mixture outside the scaffolds.

Moreover, it is important to put in evidence that in the first day of release (i.e. 24 h), a release kinetic of about zero order had been obtained: it is a well achieved target for a controlled drug release device.

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