Chapter

Foaming + Impregnation One-Step Process Using Supercritical CO_2

Antonio Montes, Clara Pereyra and Enrique Martínez de la Ossa

Abstract

Polymers are widely used in everyday life due to their properties as toughness, viscoelasticity, and the possibility to form glasses and semicrystalline structures. For that reason, it is used in mostly drug delivery systems and tissue engineering and in pharmaceutical and biomedical investigations. Foaming process allows creating porous structure into the polymer leading to scaffolds. Scaffolds are the focus of many investigations as prolonged drug delivery systems and implants or injections which are used to deliver cells, drugs, and genes into the body. Particulate leaching, freeze-drying, thermally induced phase separation, rapid prototyping, powder compaction, sol–gel, and melt molding are the main techniques in front of supercritical fluid technology to prepare scaffolds. Supercritical foaming process using CO_2 presents advantages as a high dissolution in polymers and a green process because CO_2 is nontoxic, inexpensive, and reusable. Moreover, supercritical technology allows to do an impregnation with an active substance together with the foaming at the same time. Thus active substances entrapped into scaffolds could be fabricated in a one-step green process.

Keywords: supercritical, foaming, scaffold, polymer, impregnation

1. Introduction

For decades, drug delivery systems are the focus of many investigations [1–4] because it increases the effectiveness of formulations, avoiding the first-pass effect, and reduces the drug dosage of patients, producing delayed drug delivery to increase the patient comfort. Polymers are the coating agent frequently used in pharmaceutical technology due to their properties as toughness, viscoelasticity, and the possibility to form glasses and semicrystalline and porous structures.

Microencapsulation of active substance with polymers allows to produce drug delivery systems where the release phenomena is controlled by the diffusion of the active substance through the polymer and/or the erosion and degradation of the polymer at acid or basic media. Many authors have carried out investigations to prepare controlled drug delivery systems using supercritical technology [5–8]. In most of the cases, supercritical antisolvent process (SAS) has been the chosen technology because most of the active substances are insoluble in supercritical fluids. In this process an organic solution that contains the polymer and active substance is sprayed into a chamber filled with bulk supercritical fluid. The generated microdroplets improve the mass transfer between the supercritical fluid and the solution

producing the dissolution into the solvent and the consequent solvent expansion and precipitation of particles of polymer and active substance by antisolvent effect. The result could be a coprecipitation of both compounds separately, the inclusion of particles into a matrix of polymer called composites, or the production of microcapsules with polymer coating as the active substance. In general, to avoid the separated precipitation, the ratio of polymer/active substance should be high. Moreover, operating conditions as pressure, temperature, concentration, flow rate ratios, and nozzle device have a relative influence on the final product characteristic.

However, some formulations require a long time drug delivery system as transdermal drug delivery where, for instance, hormone treatment could be carried out. Synthetic polymers, for example, polycaprolactone (PCL), polyvinyl alcohol (PVA), or polyvinylpyrrolidone (PVP), are good candidates to prepare for this kind of systems. An excellent alternative is the use of biopolymers, such as chitosan, alginate, starch, or hyaluronic acid [9].

Extended or long delay drug delivery systems are not often achieved in supercritical microencapsulation. If the active substance was placed into the pores of a polymeric porous structure, the drug release would be delayed most of the time. Thus supercritical impregnation into the pores of a polymer is an excellent alternative to prepare delay drug delivery systems.

In supercritical impregnation two processes could happen, the impregnation into the polymer pores and the foaming of polymer with the subsequent impregnated scaffold production. This fact will happen if the polymer structure is able to grow up in the depressurization step. For that many authors have carried out the foaming + impregnation one-step process and the other ones only the impregnation process. CO_2 is widely used as blowing agent because it presents properties that are nontoxic, inexpensive, and reusable and have a high dissolution in polymers.

When a polymer is put in contact with supercritical CO_2, in a first step the polymer is saturated with the gas above supercritical conditions. In a second step, the system is driven to a supersaturated state, usually decreasing the pressure or increasing temperature. This causes nucleation and relative growth of the porous cells within the polymeric matrix [10]. The fact that the polymer is under supercritical conditions alter physical properties as melting point and heat, glass transition and crystallization temperatures, crystallization rate, and swelling or foaming processes, among others, could be occasioned [11–13].

In general, as a solvent penetrated the polymer, it induced swelling and consequently facilitated the mobility of the chains, allowing reorientation of the chains to form the more thermodynamically favorable crystalline state and reducing the crystallization and melting temperatures [14].

Moreover, other authors conclude that crystallization rate of polymer-CO_2 depends only on the local degree of swelling inside the amorphous regions and the degrees of crystallinity itself [15].

Campardelli et al. [9] investigated the pore formation of PCL under CO_2 at 100–200 MPa of pressure and 35–40°C of temperature; due to a higher temperature, the polymer was melted. Process time was varied between 4 and 8 h. They concluded that formation of pores and thus the foaming of the polymer were only favored at 8 h when 100 MPa was used, but at higher pressures the foaming is produced independent of processing time. As pressure increases a regular pore structure was obtained with lower average pore diameter. However, as the temperature increases, the polymer swells more, forming a single structure, sticking polymer granules.

Thus polymer foaming could be achieved in some operating conditions producing scaffolds. The inclusion of active substance in these scaffolds is the focus of many new investigations. So when a polymer is going to be impregnated, it should

be taken into account if the foaming process will carry out to produce scaffolds or the impregnation of the active substance happens mostly on the polymer surface.

2. Supercritical CO_2

A supercritical fluid is a substance above its critical temperature and pressure. A typical pressure-temperature phase diagram is shown in **Figure 1**. At this condition the fluid has unique properties as diffusivities that are two orders of magnitude larger than those of typical liquids, resulting in higher mass transfer rates. Moreover this state presents many exceptional characteristics, such as singularities in compressibility and viscosity and diminishing difference in liquid and vapor phases, among others. It is a good candidate to do extraction or impregnation processes because density can be adjusted continuously by altering the experimental conditions of temperature and pressure so solvent power and selectivity can be tuned.

The requirement that should fulfill the supercritical fluid is on the one hand low danger and on the other hand the relative low cost. In this sense CO_2 is GRAS solvent, noninflammable, nontoxic, and gaseous at room temperature which makes the separation process easy. Besides it does not present a high cost and presents relative mild conditions of its critical point (31.1°C and 71.8 bar), allowing the thermolabile solute processing.

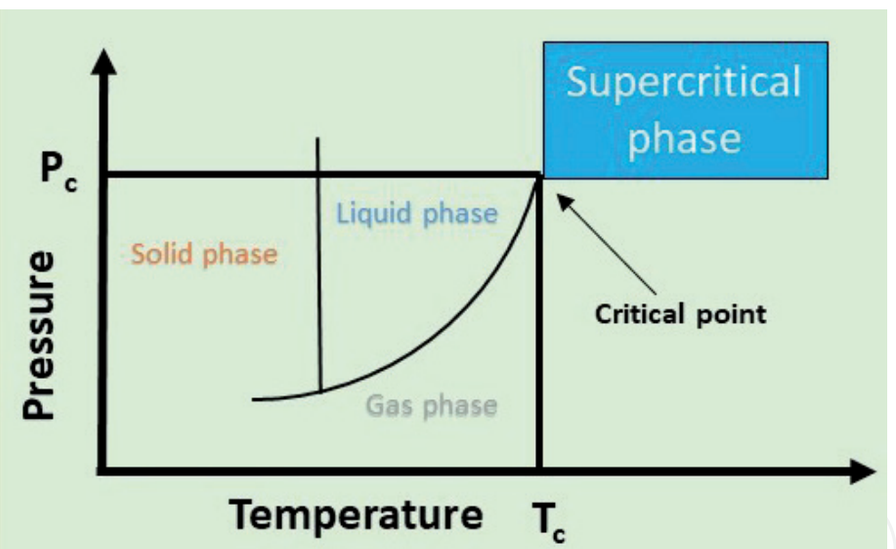

Figure 1.
Pressure-temperature phase diagram.

3. Scaffold fabrication

During the past two decades, biomedical research has advanced extensively to develop potentially applicable scaffolds. Several methods are used decades ago to manufacture these porous structures.

The solvent-casting particulate leaching (SCPL) technique is a standard method to produce polymer-based scaffolds. A polymer is dissolved in an organic solvent that contains mainly salts, with specific dimensions. Then, the mixture is shaped into a three-dimensional mold to produce a scaffold. Thus, when the solvent is removed by simple evaporation, it creates a structure of composite material consisting of the particles together with the polymer. At the end, particles are dissolved in a bath leaving behind a porous structure. In this way, Sola et al. fabricated

innovative 3D porous structures to mimic the bone marrow niche in vitro using polymethyl methacrylate (PMMA) and a flexible polyurethane (PU) and NaCl, as an efficient porogen [16].

The preparation of porous structures from a thermoplastic polymer melt is a convenient route because of the production of scaffolds of many shapes and sizes reproducibly with the use of molds and without involving any solvents. These techniques typically include compression molding, extrusion, and injection molding. Scaffolds of any desired shape could be created by simply changing the mold to use for clinical applications. Moreover, various solid fillers as bioactive molecules could be employed as additives. However, the use of high molding temperatures could degrade and inactivate the biodegradable polymer or the impregnated bioactive molecules [17].

In freeze-gelation method, the porous structure is generated during the freeze of a polymer solution, and then the solvent is extracted by a non-solvent, or the polymer is gelled under the freezing condition. Thus, the porous structure destruction would be avoided during the subsequent drying stage. Porous PLLA, PLGA, chitosan, and alginate scaffolds were successfully fabricated with this method [18].

Various porous biodegradable scaffolds with these polymers have been also fabricated by thermally induced phase separation (TIPS) technique to be used in tissue engineering and drug delivery [19–20]. This technique is based on changes in Gibbs free energy to induce the demixing of a homogeneous polymer solution to obtain a multiphase system [21]. Highly porous scaffolds of biodegradable PCL have been fabricated by this method [22]. Even PLLA scaffolds with hydroxyapatite as filler were successfully fabricated by Ghersi et al. [23].

Three-dimensional printing (3DP) is another method to produce scaffolds for tissue engineering. In 3DP method the solid is created by the reaction of a liquid selectively sprayed onto a powder bed. This is a versatile method to produce scaffolds for tissue engineering [24].

In robocasting process the object is built by printing the required shape layer by layer. For that, a filament of a paste-like material is extruded from a small nozzle while the nozzle is moved across a platform. Many authors have combined this method with sol–gel synthesis, mixing precursors in an aqueous medium. The resulting gels are used to print scaffolds by robocasting. Houmard et al. fabricated in this way highly porous calcium phosphate (CaP) scaffolds for bone-tissue engineering using calcium nitrate tetrahydrate and triethyl phosphite precursors [25].

Besides, several reports on the fabrication of porous scaffolds using sol-gel technique are found in the literature [26–32].

Scaffold could be fabricated using supercritical CO_2 as blowing agent, avoiding the use of organic solvent, and thus the presence of solvent residue in the final product due to tradition processes tested until now does not allow the complete removal of the organic solvents involved in the starting solutions, avoiding high temperatures and 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 [33].

In supercritical CO_2 foaming, the polymer is exposed to carbon dioxide, which plasticizes the polymer by reducing the glass transition temperature. Then in the depressurization step, thermodynamic instability causes supersaturation of the carbon dioxide dissolved in the polymer matrix, and hence, nucleation of cells occurs. Anyway gas foaming could not be used in polymers which have a very low affinity for CO_2 because the main requirement of process is that CO_2 can be dissolved in a sufficient amount in the polymer. So this technique is more commonly

applied to amorphous polymers excluding polymers with high crystallinity or high glass transition temperatures [34].

In this way, for instance, the PCL scaffolds produced by supercritical fluid processing had a homogeneously interconnected porous structure, and they exhibited a narrow pore size distribution. Consequently, these results indicated that the PCL scaffolds made by supercritical fluid processing offer well-interconnected and nontoxic substrates for cell growth, avoiding problems associated with a solvent residue. This suggests that these elastic PCL scaffolds formed by supercritical fluid processing could be used for cartilage tissue engineering [35].

Other authors used supercritical CO_2 in the foaming process for the formation of polyvinylidene fluoride copolymerized with hexafluoropropylene loaded scaffolds which is a material that is semicrystalline and biocompatible with a good resistance to acid environments. They concluded that a higher pressure, a lower temperature, and a longer saturation time were more favorable for obtaining uniform foam. Moreover, the average pore cell diameter in low depressurization is larger than that in rapid depressurization. Lower crystallinity and higher melting temperature were induced in the formed scaffolds [36].

Supercritical CO_2 is used sometimes as dryer to prepare scaffolds. In this way polymer solution is prepared, and then this solution is put in contact with $scCO_2$. In that moment CO_2 solubilizes the organic solvent and the scaffold is formed.

In this sense a chitosan-based scaffold for tissue engineering applications has been prepared using supercritical CO_2 as dryer. The hydrogel fabricated was subsequently processed with supercritical CO_2. The highest porosity (87.03%) was achieved at 250 bar, 45°C, and 2 h of processing at 5 g/min CO_2 flow rate [37].

However other authors investigated about a new supercritical fluid-assisted technique for the formation of 3D scaffolds to overcome the main difficulty to obtain the coexistence of the macro- and microstructural characteristics necessary for a successful application. The process consists of the formation of a polymeric gel loaded with a solid porogen, then the drying of the gel using supercritical CO_2, and the washing with water to eliminate the porogen. In this way Reverchon et al. fabricated (PLLA) scaffolds with elevated porosity (>90%) and interconnectivity and with good mechanical properties [38]. Moreover they produced scaffolds with predetermined shape and size in a relatively short time (<30 h) and without an appreciable solvent residue (<5 ppm).

Tang et al. produced porous PCL scaffolds with open and interconnected architectures based on supercritical fluid-assisted hybrid processes of phase inversion and foaming. They achieved the encapsulation growth factor in these porous scaffolds, promoting the osteogenic differentiation and thus having also a significant potential in bone tissue engineering [39].

4. Impregnation

A scaffold where a bioactive substance can be incorporated that, for instance, can control proliferation and differentiation of cells is an excellent alternative to be used in tissue engineering. In this way the function of a scaffold is not limited only as a physical support but also as a bioactive element to control cell proliferation and differentiation. Anyway, scaffold impregnation process has been mostly studied for the preparation of long time drug delivery systems, with more or less delay depending on the final purpose of the delivery.

The conventional impregnation of scaffolds uses organic solvents that dissolve the drug which is going to be incorporated into the scaffolds, but this organic solution should swell and stretch the polymer to allow the diffusion of the drug at adequate

depressurization rate. Posteriorly the organic solvent should be removed, leaving the impregnated scaffold. This method has several drawbacks, for example, residues of organic solvent in the final product need a last step to dry the scaffold, and the distribution of drug into scaffold is heterogeneous.

SCF impregnation removes all these drawbacks due to its properties as high diffusivity, low surface tension, and the ease of solvent recovery. Nevertheless, this methodology is limited by the solubility of the drug in the SCF, and the polymer can be swollen by the SCF. If these last requirements are fulfilled, a high-quality product free of residual solvents can be obtained, since no organic solvents are involved in process [40, 41]. In this process SCF is put in contact with the active substance that is going to be incorporated into the scaffolds. Then the SCF solubilizes this substance till saturation during the impregnation time. Later, in the depressurization step, the gas rapidly diffuses out of the polymer, deplasticizing it and avoiding the polymers and active substance exposition to high temperatures, which may degrade them.

CO_2 is the frequently used SCF because it is not dangerous, not toxic, not flammable, relatively cheap, and classified as a safe solvent. Anyway due to polarity limitations, it is often used as a cosolvent to increase the polarity and selectivity.

Duarte et al. prepared loaded chitosan scaffold able to sustain the release of dexamethasone using supercritical impregnation [42]. Sproule et al. [43] achieved the successful impregnation of a protein in PMMA scaffolds for biomedical applications holding unaltered the protein activity.

Campardelli et al. [9] prepared polycaprolactone/nimesulide patches using supercritical impregnation. In this work the authors achieved the foaming of the polymer in certain conditions at the same time that nimesulide is incorporated into its structure. Thus impregnated scaffolds are prepared in a one-step process for determined conditions. However in other operating conditions, foaming of the polymer is not favored, and scaffolds were not achieved.

Biodegradable PLA/PLGA foams impregnated with indomethacin in $scCO_2$ were studied by Cabezas et al. [44]. Authors observed that drug loading of foams was favored by high values of stirring rate. Moreover little pore sizes were obtained at slow depressurization rates. As it was expected, composition influenced the mechanical resistance, the PLA foams being more fragile.

Fanovich et al. studied the functionalized PCL scaffolds impregnated with natural compounds extracted from Patagonian *Usnea* lichen for tissue engineering [45]. An integrated process at high pressure for extraction/impregnation/foaming of PCL was developed. Authors concluded that the process is successful at 35°C and 15–17 MPa of CO_2 by foaming. The same researchers incorporated in a posterior work hydroxyapatite to these scaffolds, concluding that the scaffold obtained from PCL-HA with 20% of the HA shows the highest impregnation yield at 17 MPa and 35°C and subsequently also the best bactericidal effect on the tested *Staphylococcus aureus* strains [46].

The impregnation of chitosan with lactulose using supercritical fluids under various operating conditions, in order to improve the solubility of this natural polymer at neutral or basic pH, was carried out by Diaz et al. The highest impregnation yield was obtained using continuous process, 60-min contact time, 14% (v/v) of cosolvent ethanol/water (95:5), depressurization rate equal to 3.3 bar/min, 100 bar of pressure, and 100°C [47].

The impregnation of 5-fluorouracil, a chemotherapy agent, into a polymer based on D,L-lactide and glycolide was carried out at the same time to the foaming process in a one-step procedure. The possibility of regulating the rate of the scaffold degradation and the kinetics of drug release makes the usage of the copolymer more attractive for a further medical application. Venting rate is revealed to be the

most important factor affecting the probes' pore size and their morphology. Thus, slow venting rates should be used to promote small pores in order to retard the drug release from the polymeric matrix. As it was expected, vigorous stirring rates favor the contact between supercritical CO_2 and the swelled polymer, improving the impregnation process. On the other hand, the presence of glycolide enhanced the mechanical strength of the foam preventing pore collapse [48].

In the same way, Salerno et al. prepared porous PCL scaffolds containing three different drugs: 5-fluorouracil, nicotinamide, and triflusal, in order to obtain systems with controlled drug delivery capabilities. $ScCO_2$ saturation and foaming conditions were optimized to create the porosity within the samples and demonstrated that the composition of the starting PCL/drug/solvent mixtures influenced polymer crystallization, scaffold morphology, and pore structure features. Moreover, it was found that drug loading depended on both initial solution composition and drug solubility in $scCO_2$. So, in the case of triflusal that is a highly $scCO_2$-soluble drug, loading efficiency was improved by adding a higher amount of free drug inside of the impregnation vessel. The drug delivery study, as it was expected, indicated that release profiles depended mainly on pore structure and scaffold composition [49].

However, the authors observed that the control on the pore interconnectivity and pore size with this technique still needs to be improved. They proposed the use of natural plasticizers as eugenol to overcome these limitations. Thus, eugenol-containing PCL scaffolds were prepared by supercritical foaming followed by a one- or a two-step depressurization profile. Moreover these scaffolds presented antimicrobial activity preventing the attachment of Gram-positive (*S. aureus*, *S. epidermidis*) bacteria and showed good tissue integration [50].

A hybrid porous scaffold of PLGA hydroxyapatite and collagen was prepared using a supercritical CO_2 saturation technique by Zhang et al. The results showed that the pore size and porosity of the scaffold could be controlled by manipulating these process conditions. The pore size and porosity can be regulated by supercritical CO_2 saturation temperature, saturation time, and saturation pressure [51].

5. One-step supercritical foaming + impregnation process: a particular case

In our facilities PCL scaffolds impregnated with quercetin were prepared using supercritical CO_2. PCL is a semicrystalline polyester with a melting point (Tm) of 329–334 K and a glass transition temperature (Tg) of 213 K [52]. Quercetin (Q) is a flavonoid present in many fruits and vegetables [53]. This flavonoid highlights its antioxidant action, but it has different benefits as antibacterial, cardiovascular health, anti-inflammatory, and anticancer effects [54, 55]. The study was supported by an experimental design to elucidate the influence of pressure (15–30 MPa), temperature (308–333 K), and depressurization rate (0.1–20) on foaming, melting temperature, melting heat, and amount of released quercetin.

The experiments were carried out in a plant RESS250 developed by Thar Technologies [56]. PCL and quercetin were mixed physically into an aluminum foil support (ratio 50:1 PCL/Q), and it was introduced into a stainless steel vessel. Then, CO_2 was pumped to the vessel till the desired operating pressure at the same time that the temperature was adjusted is reached. A determined impregnation time was awaited, and once finished, the output valve was opened to vent the CO_2 in a range of depressurization rate of 0.1–20 MPa min^{-1}. In the SEM image in **Figure 2**, it can be seen that PCL/quercetin foamed in our facilities [56].

The generated PCL/quercetin scaffold with higher pore density and smaller pore size was achieved for higher pressure and depressurization rate and lower

temperatures (300 bar, 308 K, and 20 MPa min^{-1}). In general according to our results, the high level of temperature is recommended to obtain a pronounced effect of foaming to produce scaffold.

It was also observed that experiments done at lower pressure and temperature together with a higher depressurization rate led to a higher melting temperature. An increase of pressure and temperature leads to composite which released a higher amount of quercetin. However, depressurization rate has the opposite trend, so an increase of depressurization rate leads to a lower amount of released quercetin. These facts can be observed in the contour surface plots (**Figures 3** and **4**).

Release profiles showed that quercetin took five times longer to dissolve the same amount of quercetin into the first 8 h, demonstrating the efficacy of using PCL to control quercetin release and its possible use with other medical or pharmaceutical compounds (**Figure 5**).

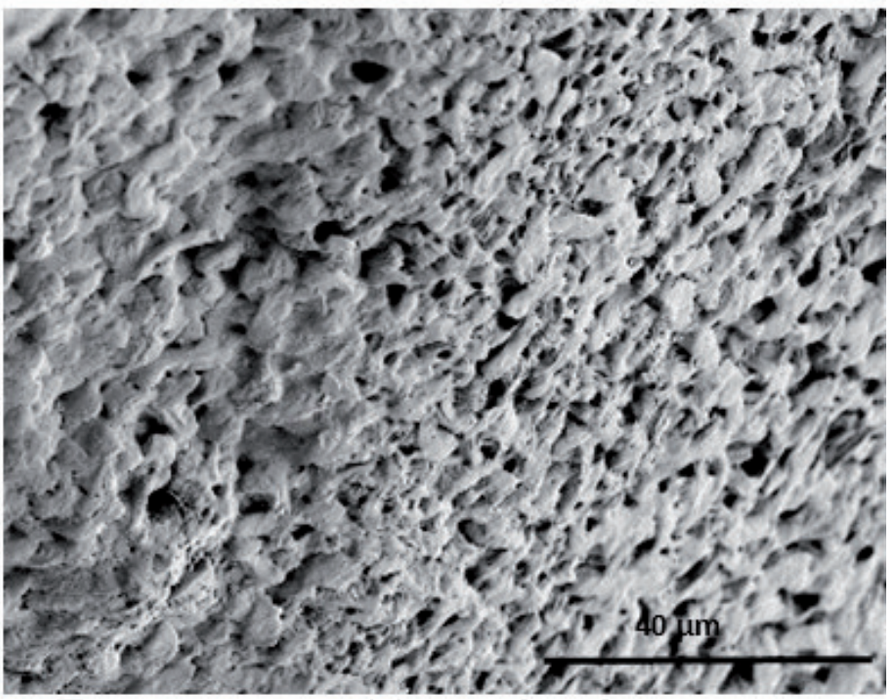

Figure 2.
SEM image of PCL/quercetin scaffold.

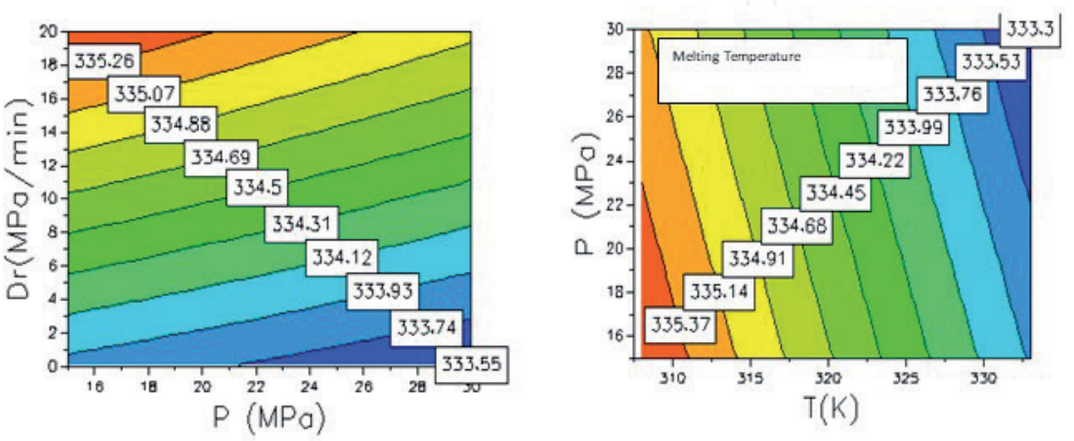

Figure 3.
Contour plot of melting temperature of PCL/quercetin scaffold.

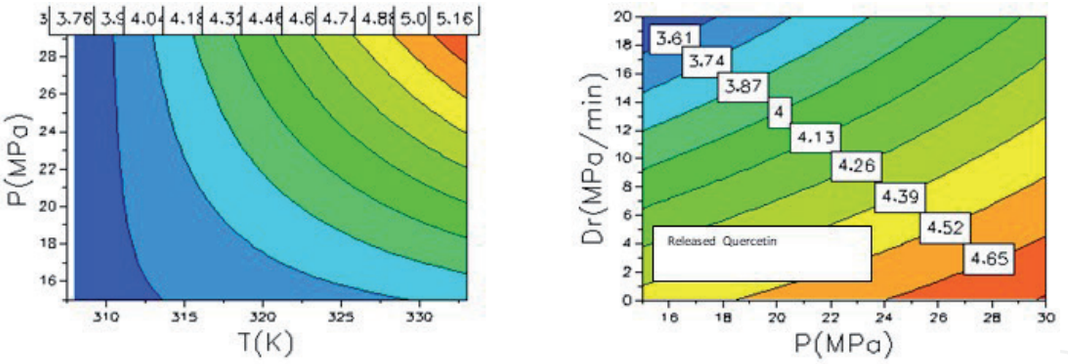

Figure 4.
Contour plot of release quercetin of PCL/quercetin scaffold.

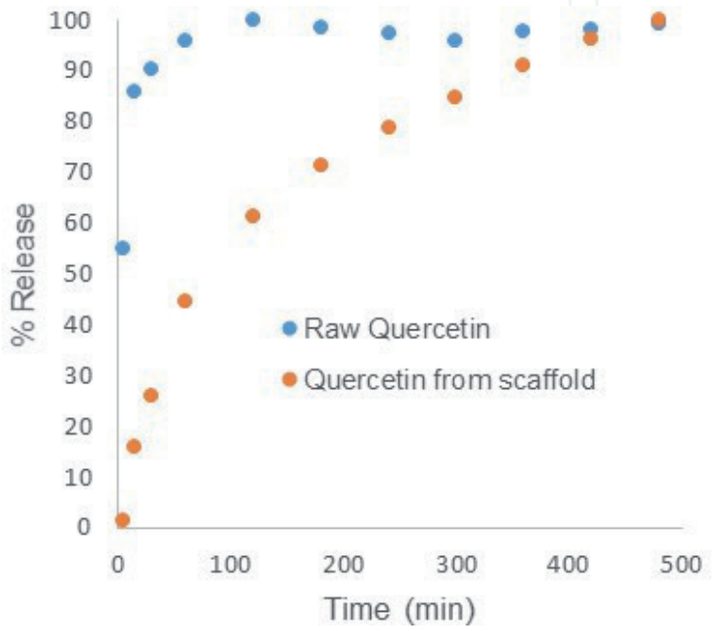

Figure 5.
Release profile from raw quercetin and PCL/quercetin scaffold.

6. Conclusions

Impregnated scaffolds are an interesting alternative to be used in pharmacology and biomedicine because scaffolds act not only as a physical support but also as a carrier of a bioactive substance with a controllable release. The possibility of regulating the rate of the scaffold degradation and the kinetics of drug release make it easy to fabricate particular drug release systems. Bone regeneration, implants, hormonal treatment, and tissue engineering applications are fields where scaffolds could be used. The way to create the porosity in the polymer originated a multiple scaffold fabrication methods based most of them in molding and removing the used organic solvent in a posterior step. Supercritical CO_2 has been used as dryer in many conventional methods as sol-gel where the solvent must be evaporated. However, in these methods supercritical CO_2 is able to remove almost the totality of used organic solvent, which requires several process steps. Another way to use supercritical CO_2 is in the gas foaming process. In this sense the porosity is created at the same time that the bioactive substance is incorporated, avoiding the use of organic solvent. Moreover these processes do not use high temperature, so the activity of the bioactive molecule would hold unaltered. Foaming process changes not only the polymer porosity but also other properties as melting, crystallization or glass transition

temperature, melting heat, and so on and thus could produce more fragile or harder polymer depending on the foaming conditions. Particularly in our facilities, polycaprolactone/quercetin scaffolds were prepared using supercritical CO_2 foaming + impregnation one-step process in an efficient way. Release profiles showed that quercetin took five times longer to dissolve the same amount of quercetin into the first 8 h where it was placed into scaffold.

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Conflict of interest

The authors declare no conflict of interest.

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