Supercritical CO\textsubscript{2} Impregnation of PCL and PCL-HA Composites

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Development of batch and semi-continuous processes for creation and impregnation of polycaprolactone (PCL) and PCL-hydroxyapatite (PCL-HA) scaffolds using supercritical carbon dioxide (scCO\textsubscript{2}) was presented. The effect of scCO\textsubscript{2} sorption on swelling, scaffold morphology and thermal behaviour of PCL and PCL-HA composites was considered for design and optimization of impregnation processes. Influence of pressure, amount of HA within the composite and the procedure for powder preparation on sorption was analysed. Natural substances with strong antibacterial activity such as thymol and usnea extract were used as impregnating substances. For both processes, moderately high pressure (17 MPa) and temperatures (35–40 °C), and low percentages of HA (10%) were favourable for creation of the microcellular PCL or PCL-HA scaffolds with satisfying impregnation yields of thymol (13%) and usnea extract (4%).

INTRODUCTION

Bioactive compounds are incorporated into solid materials to prevent and mitigate inflammation, inhibit oxidation processes, act as cleansing agents, prevent bacterial infection or to accelerate tissue regeneration. As a good solvent for a wide range of natural bioactive principles with high diffusion ability in organic matter, scCO\textsubscript{2} represents a good medium for impregnation of solid matrices with active substances soluble in this fluid. At the same time, scCO\textsubscript{2} plasticizes most of amorphous and semi-crystalline polymers lowering their glass transition or melting temperature [1,2] which allows different modifications such as incorporation of additives, formation of foams, polymer blend preparations, polymer bulk graft-modification, etc. Supercritical solvent impregnation (SSI) process employs scCO\textsubscript{2} as the carrier solvent, potential polymer swelling/plasticizing and foaming agent [3–5]. Moreover, it enables working at low operating temperatures and with thermolabile and hydrophobic substances as well as fast and complete solvent removal from the final product [4]. Tuning of CO\textsubscript{2} pressure and temperature, contact time, and depressurisation rate provides control of incorporation yields and depth of penetration of active substances into a solid matrix simultaneously tailoring chemistry and morphology the final product.

The SSI can be designed as a batch or semi-continuous process. In a batch process, solid substrate and an impregnating substance are placed in the same vessel without or with scCO\textsubscript{2} circulation provided by stirring or circulating pump. Semi-continuous impregnation implies
dissolution of an active substance in scCO₂ in a separate vessel followed by supercritical solution flowing through a fixed bed of substrate placed in an adsorption vessel. If there is a demand to impregnate a natural extract into the selected carrier using scCO₂ it is reasonable to couple supercritical fluid extraction (SFE) and SSI. The coupled SFE-SSI process excludes intermediate decompression step and enables direct use of the scCO₂-extract solution leaving the extractor vessel for impregnation [6]. This strategy is thus advantageous for time and energy savings as well as for minimization of the loss of the extract in the tubes, vessels and exchangers of the equipment.

Predictive knowledge of scCO₂ sorption in polymer and its effect on swelling and phase transitions is fundamental for design of supercritical-based processes for creation of porous scaffolds [6-8]. ScCO₂ solubility, as maximum amount of the gas dissolved in the polymer without causing phase separation [9], determines the extent of the plasticization effect on the polymer, while its diffusivity affects bubble nucleation and cell growth, both influencing final density of the polymer foam [10].

In this work, batch SSI and semi-continuous SFE-SSI processes were used to create and impregnate PCL and PCL-HA scaffolds which have high importance for biomedical applications, [11,12] with thymol and usnea lichen extract previously proven for strong antibacterial effect against staphylococci including methicillin resistant strains [13,14]. Design and optimization of impregnation processes regarding morphology and impregnation yields were based on thermodynamic measurements under high CO₂ pressures. The effects of pressure, procedure for HA powder preparation and its amount on polymer sorption and thermal properties were investigated to find out optimal parameters of the aforementioned impregnation processes.

MATERIALS AND METHODS

Granules of the PCL (CAS 24980-41-4, Sigma Aldrich, Germany) with density of 1.145 g/cm³ and commercial CO₂ (99.8% purity, Yara GmbH, Brunsbüttel, Germany) were used for experiments. Precipitation method [15] with stirring and ultrasonic irradiation treatment was used to obtained two types of hydroxyapatite filler particles denoted as M (mean particle size of 10 μm; specific surface area of 15 m²/g) and S (mean particle size of 5 μm; specific surface area of 33 m²/g), respectively. Nano-HA powder, HA(N) (mean particle size < 1 μm; specific surface area of 70 m²/g) was obtained by López Macipe [16]. The obtained HA powders were added to a solution of PCL in acetone (1:10) and dispersed with the Ultra Turrax T25 (IKA, Germany) at 20,000 rpm for 10 min. Nominal content of HA added to PCL/acetone solution was 10% or 20% with respect to the PCL weight. Thymol (purity<99%, Sigma Aldrich, Germany) was used as one impregnation agent. Usnea lethariiformis lichen (Ushuaia, Tierra del Fuego, Argentinian Republic) was used for SFE of antibacterial substances.

A high-pressure view chamber (Pₘₐₓ = 35 MPa, tₘₐₓ = 120 °C, Eurotechnica, Germany) with a CCD camera was used for the visual monitoring and quantification of swelling extent of disc-shaped samples placed in glass recipients and exposed to scCO₂ at 10-30 MPa and 35-40 °C [17]. The images of two-dimensional projection of the rotationally symmetric sample were recorded for 24 h using IC Capture 2.1 software. Relative volume change denoted as swelling extent (S_w) was calculated using Eq. (1):

\[ S_w (%) = \frac{V_f - V_0}{V_0} \cdot 100 = \left( \frac{H}{H_0} - 1 \right) \cdot 100 \]  

(1)
where, $V_0$ and $V_t$ are the samples' volumes at $t=0$ (ambient conditions) and $t>0$ at given CO$_2$ pressure and temperature, respectively. $H_0$ and $H$ denote sample heights at $t=0$ (ambient conditions) and $t>0$ at given CO$_2$ pressure and temperature, respectively. Discs' diameters are constant and equal to glass recipient diameter. Heights were determined by image processing software Image-Pro Plus 6.0.

A magnetic suspension balance ($p_{\text{max}} = 35$ MPa, $t_{\text{max}} = 120$ °C, Rubotherm, Germany) was used for scCO$_2$ sorption measurements [17]. The sample weight under actual conditions of pressure is transmitted to a microbalance outside the autoclave by a magnetic suspension coupling and recorded. The recorded balance readings in time were corrected for the change in buoyancy that occurs due to swelling. The mass gain ($M_t$) under pressure can be determined by knowing the exact volume of the sample at a certain time $t$ ($V_t$), the initial weight of the sample at atmospheric pressure ($w_0$) and the density of CO$_2$ ($\rho_{\text{CO}_2}$).

$$M_t = (w_t - V_t \rho_{\text{CO}_2}) - w_0$$

A low temperature Tian-Calvet differential scanning calorimeter BT2.15 (Setaram, France) [17] was used for high pressure differential scanning calorimetry (HP-DSC) measurements. High pressure crucibles made of Inconel 625 ($V=3.6$ cm$^3$, $p_{\text{max}}=60$ MPa, $T_{\text{max}}=500$ °C) were used for measurements at pressures in the range of 4.6-17.0 MPa. The sample was heated at the rate of 0.10 °C/min from 25 °C to 85 °C. An empty, hermetically sealed stainless steel pan was used as a reference. Melting point and enthalpies of indium were used for temperature and heat capacity calibration. Fusion enthalpy ($\Delta H_m$) calculated using Calisto Data Acquisition software was used to determine degree of crystallinity ($\chi_c(\%) = (\Delta H_m / \Delta H_m^0) \cdot 100$). Heat of fusion value of 100% crystalline PCL ($\Delta H_m^0$) of 135.31 J/g was a literature data [18].

Batch SSI of samples with thymol was realized in a high-pressure view chamber using a static method previously described elsewhere [14]. Thymol was placed at the bottom of the vessel in a glass container. Polymer samples were placed in a wire mesh basket above the thymol. The experiments were carried out at pressures of 10-30 MPa and temperatures of 35 °C and 40 °C for 2 h. Substrate to thymol mass ratio was 1:1. Decompression rate was 0.5 MPa/min [10]. Mass of the impregnation substance ($m_{IS}$) was determined gravimetrically as differential mass of the impregnated sample and initial mass of the polymer ($m_p$). The impregnation yield (I) was calculated from Eq. (3):

$$I(\%) = \frac{m_{IS}}{m_{IS} + m_p} \cdot 100$$

The unit HPEA500 (Eurotechnica, Germany) described elsewhere [6] (Fig. 1) was used for lichen scCO$_2$ extraction and extract incorporation into the polymer samples. The procedure was characterized by two variables of time, $t_1$ and $t_2$, where $t_1$ is the time of continuous extraction-adsorption in a single passing mode at given conditions (extraction: 30MPa/40 °C and adsorption: 17 MPa/35 °C or 30 MPa/40 °C) and $t_2$ represents the time of recycling of the solution at the adsorption conditions through both extractor and adsorption vessel.

Scanning electron microscopy (FE-SEM, Mira3 XMU TESCAN a.s., Brno, Czech Republic) at accelerating voltage of 10 kV was used to analyse scaffold morphology. The scaffolds were coated with a thin layer of Au/Pd (85/15), using a sputter coater (POLARON SC502, Fisons Instruments, Ipswich, UK) prior to the analysis. Apparent foam density...
(\(\rho_{\text{foam}}\)) was determined by the water displacement method in accordance with ASTM D792-0022. Density of water at 23.3±0.5 °C is 997.3±0.2 kg/m³. Porosity was calculated using Eq. (4) [19].

\[
\varepsilon(\%) = \left(1 - \frac{\rho_{\text{foam}}}{\rho_{\text{PCL}}}\right) \cdot 100
\]  

(4)

\[\text{Figure 1: Setup for a developed process that integrates extraction, impregnation and foaming}\]

\[\text{RESULTS AND DISUSSION}\]

Sorption of scCO\(_2\) in PCL and PCL-HA composites was related to their swelling behaviour at moderately high to high pressures (10-30 MPa) and mild temperatures (35 or 40 °C) prior to their impregnation. Effects of CO\(_2\) pressure, HA\% and the procedure for HA powder preparation on scCO\(_2\) solubility and swelling extent of the samples is presented in Fig. 2. Pressure increase had positive effect on scCO\(_2\) solubility and swelling extent of PCL. Decreased scCO\(_2\) sorption in the case of composites with 10% or 20% of N and M powders (N10, M10 and M20) in comparison to composite containing 10% of S powder (S10) might be due to more intimate contact and even dispersion of the filler both resulting in a decrease of free volume within the polymer which is otherwise available to the dissolving gas.

\[\text{Figure 2: ScCO}_2\ \text{solubility (qCO}_2\) in the (a) neat PCL and (b) PCL-HA composites, (c) swelling extent of PCL and PCL-HA composites}\]
Higher HA amount (20%) in the composite M20 resulted in somewhat decreased scCO$_2$ sorption and swelling extent compared M10. This could be due to the increased amount of the filler which doesn’t change its volume during scCO$_2$ sorption and acts like an obstacle to the mass transfer and gas sorption [20]. The influence of pressure and HA amount on microstructure of the scaffolds obtained after swelling experiments was analysed by SEM. Microcellular structure was observed in all the cases (Figs. 3a and 3c). The PCL scaffolds obtained at 13 MPa and higher pressures had a homogeneous pore distribution, satisfactory porosity (72-79%) [7] and average pore diameter (d$_{avg}$) of 307±11μm (Fig. 3a and 3b) which is in accordance with requirements for proper bone tissue ingrowth and vascularization [29]. Addition of 10-20% HA resulted in formation of scaffolds with smaller pores (d$_{avg}=193±15μm$) (Fig. 3c) and similar porosity (72-76%) (Fig. 3d) compared to neat PCL scaffolds at the same pressure/temperature conditions. HP-DSC was applied to quantify plasticizing effect of scCO$_2$ sorption. Determined peaks of melting temperature (T$_{m}$), fusion enthalpies ($\Delta H_m$) and corresponding degree of crystallinity ($\chi_c$) are given in Table 1. Pressure increase resulted in shift of T$_{m}$ and $\chi_c$ toward lower values reaching minimum values at 10 MPa (34.4 °C and 34.0%, respectively). Further pressure increase led to slight increase of the T$_{m}$ and $\chi_c$. Besides hydrostatic pressure effect, shifting of the T$_{m}$ and $\chi_c$ to the higher values, has been recently explained by two more phenomena: lamellar thickening due to the enhanced chain mobility upon dissolution of dense CO$_2$ into the polymer which leads to crystallization (at p > 3.6 MPa) [1,27], and the possible scCO$_2$ extraction of the low molecular weight fractions, that sometimes act as plasticizers inside the polymer matrix [28]. Presence of 10-20% had no significant influence on T$_{m}$ of PCL at the same pressure (17 MPa) (Table 1). Thermal properties of the N10 and M10 samples were similar to PCL supporting the previous assumptions on better dispersion of the filler and more intimate contact with the polymer matrix. The lowest $\chi_c$ of S10 and M20 composites was in accordance to the previous assumption on uneven distribution of the filler and/or its week contact with matrix.

Figure 3: (a) Average pore diameter and (b) porosity of PCL scaffolds, (c) average pore diameter and (d) porosity of PCL-HA scaffolds
PCL-HA composites M10 and M20 were chosen for fabrication of antibacterial scaffolds due to the simplicity of their preparation and satisfactory thermal properties during exposure to scCO\textsubscript{2}. For batch SSI of the samples with thymol, short contact time (2 h) was chosen due to the previously proven high sorption rate and plasticizing profile of the PCL at 35-40 °C [10,17] as well as to minimize negative effects of the higher amount of adsorbed thymol on the scaffold morphology [14].

<table>
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<tr>
<th>Table 1: Thermal properties of PCL and PCL-HA</th>
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<tr>
<td>p (MPa)</td>
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</tr>
<tr>
<td>PCL</td>
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<tr>
<td>0.1</td>
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<tr>
<td>4.6</td>
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<td>9</td>
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<td>17 S10</td>
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<td>17 M20</td>
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Impregnation yield decreased with scCO\textsubscript{2} pressure increase (Fig. 4a) due to a higher affinity of thymol to scCO\textsubscript{2} than to the polymer phase at higher pressures (especially above 17 MPa) [14]. All the investigated composites showed lower SSI yields (7.0-16.4%) in comparison to the neat PCL at the same conditions (Fig. 4b). As expected, the difference in the SSI yields for the composites and neat PCL was more noticeable at the lower pressure, 17 MPa (due to the lower affinity of thymol to the scCO\textsubscript{2}). The highest composite impregnation yield of M10 justified again its suitability for the production of functionalized scaffolds. Increased amount of filler (20%) had negative effect on the SSI which was in accordance with its lower scCO\textsubscript{2} sorption (Fig. 2b).

**Figure 4:** Impregnation of (a) neat PCL at 40 °C and (b) PCL-HA with thymol using batch SSI

**Figure 5:** Influence of loaded thymol (%) on morphology of PCL and PCL-HA scaffolds (scale bar=500 μm)

Scaffold M10 more was more suitable for SSI with thymol then neat PCL scaffold due to lower impact of loaded thymol on its morphology, especially at lower pressure (Fig. 5). The average pore diameters of the M10 scaffold
impregnated with thymol at 17 MPa (198±73 μm) and non-impregnated M10 scaffold obtained at the same pressure (204±75 μm) were practically the same. On the contrary, average pore diameter of the PCL scaffold impregnated with thymol at 17 MPa was 54% larger (455±95 μm) compared to the non-impregnated PCL scaffold at the same pressure (296±78 μm). The SSI at higher pressure (30 MPa) had more pronounced negative effect on the M10 and PCL scaffold morphology resulting in 80% and 100% larger pores (257±144 μm and 621±347 μm), respectively, than observed for non-impregnated samples at the same pressure (Fig. 5). Therefore, for the batch SSI of PCL with thymol, moderately high pressures (13-17 MPa) and 10% percentage of HA (10%) were suggested for production microcellular scaffolds (200-300 μm) with satisfactory high impregnation yields (12-18%). To optimize the process regarding impregnation yield and processing time, pressure and temperature in the adsorber were increased and flushing was excluded. Pressure and temperature increase in the adsorber had a positive effect on the impregnation of M10 and adverse effect on M20 scaffold (Table 2). This might be due to a more inhomogeneous filler distribution. Proper porosity of M10 scaffold (72-76%) was achieved after exposure to scCO2 at 17 MPa and 35 °C or 30 MPa and 40 °C (Fig. 3).

Table 2 Experimental conditions and results of integrated SFE-SSI process (scale bar=500μm)

<table>
<thead>
<tr>
<th>#Run</th>
<th>M (%)</th>
<th>pads (MPa)</th>
<th>tads (°C)</th>
<th>Procedure</th>
<th>I (%)</th>
<th>ε (%)</th>
<th>SEM</th>
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<td>1</td>
<td>0</td>
<td>17</td>
<td>35</td>
<td>t1: 2 h</td>
<td>2.8</td>
<td>40</td>
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<td></td>
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<td>t2: 1 h</td>
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<tr>
<td>2</td>
<td>10</td>
<td>17</td>
<td>35</td>
<td>t1: 2 h</td>
<td>4.1</td>
<td>67</td>
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<td>t2: 1 h</td>
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<tr>
<td>3</td>
<td>10</td>
<td>30</td>
<td>40</td>
<td>t1: 2 h</td>
<td>5.7</td>
<td>75</td>
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<td>t2: 0 h</td>
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<td>20</td>
<td>17</td>
<td>35</td>
<td>t1: 2 h</td>
<td>5.9</td>
<td>49</td>
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<td>5</td>
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<td>t1: 2 h</td>
<td>1.7</td>
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<td>t2: 0 h</td>
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Loading of usnea extract had no significant adverse effect on the scaffold porosity (67% and 75%, respectively) (Table 2). Besides, conditions for runs 2 and 3 gave satisfactory high impregnation yields (4.1% and 5.7%, respectively) for having strong antibacterial effect [21]. In summary, optimal conditions for creation and impregnation of microcellular PCL scaffolds
with usnea extract using SFE-SSI regarding scaffold morphology and impregnation yield implied (a) preparation of PCL-HA with 10% nominal content of HA with solvent casting technique, (b) processing conditions: 2 h of extraction (extractor: 30 MPa/40 °C; adsorber: 17 MPa/35 °C) followed by 1 h of rinsing at 17 MPa (both vessels) (run 2), or (c) just 2 h of extraction and adsorption at 30 MPa/40 °C without rinsing (run 3).

CONCLUSION

An approach in design and optimization of processes for supercritical impregnation and foaming of polymers based on thermodynamic measurements under elevated CO₂ pressure was presented. Moderately high pressure and temperatures (17 MPa, 35-40°C), low nominal HA content (10%) and relatively short contact time (2 h) were suggested for production of microcellular PCL scaffold (200-300 μm) with sufficient loads of thymol (13%) and usnea extract(4%). The aforementioned processes hold great potential for functionalization of polymeric materials and fabrics for tissue engineering, wound dressing, pharmaceutical and active packaging applications.

REFERENCES