IMPREGNATION OF BOTH POROUS AND NON-POROUS POLYMERIC SYSTEMS AIDED BY $SCCO_2$. FOAMS PRODUCTION.

C. Domingo[1*], A. Fanovich[1], J. Fraile[1], E. Loste[1], J. Gómez-Morales[1], C. Elvira[2], J. San Román[2], E. Larroz[2], J.A. Ayllón[3], P. Subra[4]

[1]Instituto Ciencia de Materiales de Barcelona, CSIC, Campus de la UAB, 08193 Bellaterra, Spain, conchi@icmab.es

[2]Instituto de Ciencia y Tecnología de Polímeros, CSIC, Juan de la Cierva 3, 28006 Madrid, Spain.

[3]Dept. Química, Universidad Autónoma, Campus UAB 08193 Bellaterra, Spain.

[4]Laboratoire d'Ingenierie des Matériaux et des Hautes Pressions, C.N.R.S., Institut Galilée, Université Paris Nord, 19 Avenue Jean Baptiste Clément , 93430 Villetaneuse, France.

Polymeric materials, in general, have a very limited solubility in $SCCO_2$, although high-pressure CO_2 usually interact with polymeric networks acting as a swelling agent. This is an interesting feature used for different processes, such as plasticization at a low temperature, purification, foaming and impregnation. The foaming of nonbiodegradable PMMA and biodegradable PLA was studied. The results presented here are part of an ongoing work aimed to prepare foamed polymer nanocomposites filled with HAP. Those are dense materials with the mechanical characteristics needed to replace bone, and the biological characteristics required to encourage the body to produce new bone. Preliminary work has shown that conventionally prepared nano-HAP particles can be converted into carbonated apatite, that closely matches the chemical composition of bone mineral, by using a SCF-route.

INTRODUCTION

Many of the polymer processing applications using supercritical fluid (SCF) technology exploit the fact that carbon dioxide (CO_2) is a good swelling agent and plasticizer while being a poor solvent. One of these new applications is related to the formation of porous polymers [1,2]. Traditionally, microcellular polymers are developed by dissolving the polymer in an appropriate hydrocarbon solvent and quenching the temperature to induce phase separation. Conventional processes often require large volumes of porogenic organic solvents. $SCCO_2$ is an excellent non-solvating porogenic diluent for the formation of well defined porous polymers. Using this process, the pore growth occurs during the CO_2 venting procedure. Pores nucleate in the polymer-rich regions and grow via diffusion of CO_2 from polymer-rich regions into the pores and boiling of liquid CO_2 at reduced pressures. The pore structure grows until polymer vitrification occurs. Pressure-induced phase separation is used to produce micro or nanoporous polymers for applications related to foams, fibers, membranes, dielectric materials and scaffolds. A porous polymer can be easily impregnated with solutes presenting certain affinity for the matrix. Additionally, the depression in the glass transition temperature of polymers in presence of $SCCO_2$ facilitates the mixing of filling materials (organic or inorganic) into the plasticized polymer.

Results presented here are part of an ongoing investigation, the final objective of which is to design a simultaneous SCF plasticization/mixing/impregnation/foaming method to produce porous polymers, filled with a reinforcing material and an active compound, and suitable for use as a bone cement for skeletal repair.

The type of materials under investigation are inert biocompatible polymers (polymethylmethacrylate, PMMA) and degradable materials (polylactic acid, PLA) and a bone inorganic analogous component (hydroxyapatite, HAP). We have investigated the use of $SCCO_2$ as a foaming agent in a non-reactive (PMMA and PLA) and a reactive (PMMA) processing. The non-biodegradable PMMA is a rigid, transparent, and reasonably tough polymer that has been employed as a bone cement for a long time. Bioabsorbable polymers (PLA) are extremely useful because they obviate the necessity for a subsequent surgical operation to remove the device after the healing process has been completed. Foamed polymer nanocomposites filled with HAP are dense materials with the mechanical characteristics needed to replace bone, and the biological characteristics needed to encourage the body to produce new bone [3]. Finally, orthopedic surgical procedure carries the risk of bacterial infection. Sustained high local concentrations of antibacterial compounds are required to minimize the occurrence of infection. Active drug compounds can be added to the foamed polymer nanocomposites transforming them into a drug delivery systems. $SCCO_2$ serves both as a foaming agent for the polymer and as a volatile solvent for additives to be impregnated. In this work, cholesterol was used as model compound of low water solubility active drugs for impregnation.

EXPERIMENTAL

Materials

Characteristics of polymers used in this study are listed in Table 1. PMMA (supplied by Plexidon) was the only commercial polymer. PMMA/TMA is a copolymer of methylmethacrylate (MMA) an tritonmethacrylate (TMA), prepared by suspension polymerization of MMA in an aqueous solution of poly(vinyl alcohol), using benzoyl peroxide (BPO) as a radical initiator, and adding to the medium 2 wt% of TMA and 25 wt% of xylene (Xy). PLA, of low molecular weight, was prepared by direct polycondensation of lactic acid using p-toluenesulfonic acid as a catalyst and xylene as a solvent.

Table 1. Characteristics of polymers employed. M_w: molecular weight.

Polymer	Composition [wt%]	M_w [Dalton]	Habit
PMMA	$95_{PMMA}\,4_{MMA}1_{BPO}$	500 000	spherical
PMMA/TMA	$90_{PMMA}2_{MMA}1_{TMA}6_{Xy}1_{BPO}$	45 000	spherical
PLA	100_{PLA}	22 000	powder

Needle-like nanometric HAP particles were synthesized by combining aqueous solutions of $CaCl_2$ (0.05 M), Na_3citrate (0.2 M) and Na_2HPO_4 (0.06 M). The resulting solution was heated at 373 K under refluxing conditions for 1 h. The obtained suspension was centrifuged and the precipitate freeze dried. Cholesterol (Fluka >99 wt%) was used as a solute for impregnation.

Characterization

Purity and composition of processed solid samples were determined by proton nuclear magnetic resonance (^{1}H NMR, Bruker ARX-300 NMR spectrometer, deuterated chloroform 300 MHz). Micrographs of polymer powders were obtained by using a scanning electron microscope (SEM, JEOL JSM-840). HAP powders were characterized by X-ray diffraction (XRD, Rigaku Rotaflex RU-200B, Cu Kα radiation, 50 kV) over the range $20 < 2\theta < 55^o$.

Apparatus and procedure

Non-reactive behavior of polymeric and inorganic materials upon contact with SC-CO_2 was studied in a high-pressure bench-scale equipment (Fig. 1). A one-pass flow technique was used for polymer purification, foaming and impregnation experiments. Same equipment was used to prepare carbonated HAP.

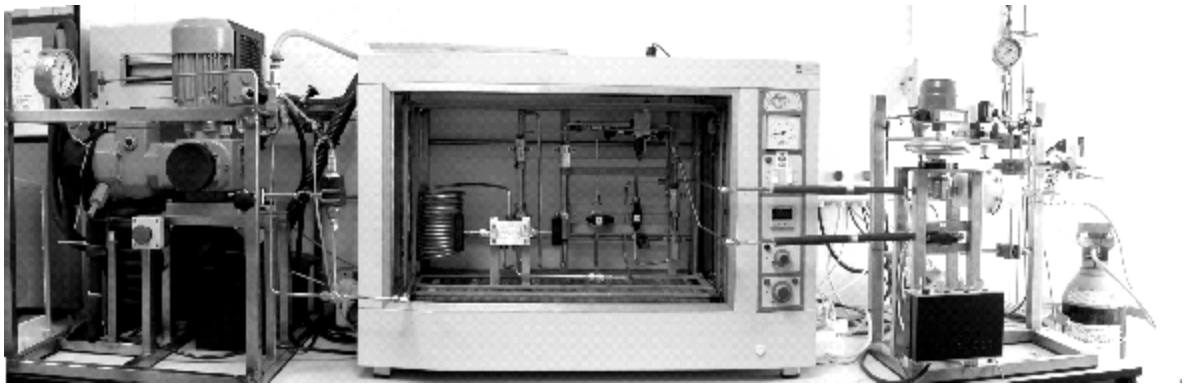


Figure 1. Continuous flow equipment wit tubular reactors (10 ml). CO_2 was compressed to the operating pressure with a membrane pump (Lewa EK-M-210). Reactors were heated in an oven air (Selecta). System pressure was controlled by means of a back pressure regulator (Tescom 26-1761).

A typical foaming experiment started by charging one of the reactors with ~1 g of polymer. The system was then heated and pressurized to the selected working conditions. To purify the polymer, the CO_2 was allowed to flow through the vessels for 1-2 h, . At the end of the flow period, the reactor was isolated from the CO_2 flow by closing the corresponding valves. Reactor pressure was maintained for 20 h. Finally, the system was depressurized controlling the rate of pressure release (within 5 sec and 1 h for a pressure drop of 20 MPa). A similar procedure was followed for the foaming/impregnation experiments, but adding cholesterol to a reactor and polymer beads to another one connected in series to the first one.

The general procedure for the incorporation of carbonate ion into the nano-HAP structure was similar to the one described for polymer treatment. System pressure and temperature were 25 MPa and 333 K, respectively. The CO_2 was firstly allowed to flow through the vessel for 1-2 h, in order to eliminate the excess of citrate. Next, a non-flow step of 20 h was performed, and, finally, the system was depressurized to recover the sample.

PMMA reactive polymerization in $SCCO_2$ was performed in a batch-stirred equipment (Fig. 2). In a typical experiment, the autoclave was charged with 10 g of MMA monomer, 0.15 g of BPO and 60 ml of CO_2 at 25 MPa and 338 K and stirred at ~1000 rpm during 24 h.

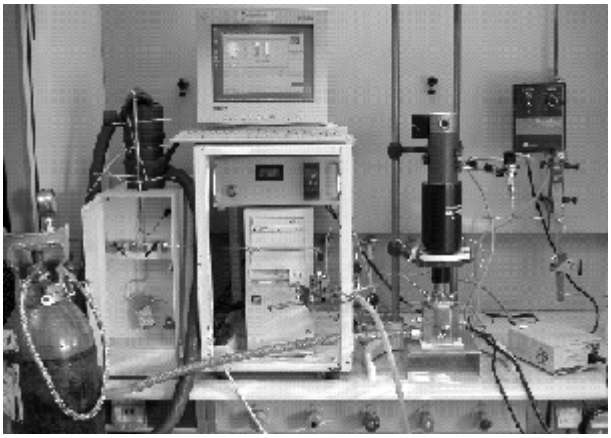
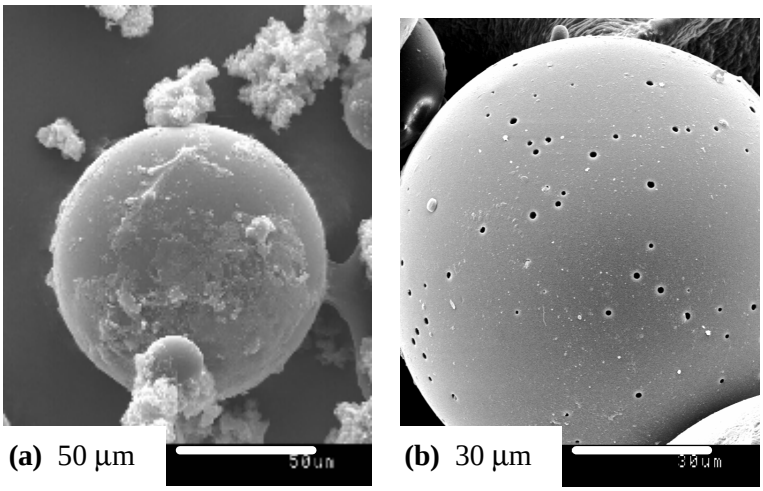


Figure 2. High pressure stainless steel autoclave (70 ml) with two sapphire windows (TharDesign) fitted with a vertically mounted impeller stirrer (DynaMag, 2500 rpm). The CO_2 was pressurized with a syringe pump (TharDesign, SP240). The reactor was heated using resistances. Pressure release was controlled by opening a needle valve.

RESULTS AND DISCUSSION

For medical and pharmaceutical use, polymers should be purified of organic impurities such as solvents, additives, monomers, oligomers, etc. A $SCCO_2$ process for purification was carried out as a first step in the nonreactive process, without adversely affecting the morphological and physical characteristics of the polymer. ^1H MNR analysis showed that residual monomer, initiator and xylene (when present) disappeared from the samples. Secondly, the effect of $SCCO_2$ on the structure and properties of powdered polymers was examined. The swelling and foaming behaviour of PMMA films in presence of $SCCO_2$ has been widely described [2,4]. Generally, a uniform distribution of macroporous throughout the polymer matrices is found, but a solid film of polymer (skin layer) is observed over the majority of the sample surface. Skin layers result from rapid diffusion of the dissolved gas from the surface. In this work, we have used PMMA beads instead of a polymer film. SEM pictures have shown that no external porosity was apparent in the beads after depressurization (Fig. 3a), although nonaccessible internal porosity is likely present. On the contrary, PMMA polymer modified with TMA macromonomer have shown external porosity clearly evidenced by visual observation of SEM micrographs (Fig. 3b). Possibly, the presence of the macromonomer reduced polymer mobility and partially prevented the formation of the outer skin. Xylene could also induce pore formation in the outer shell.

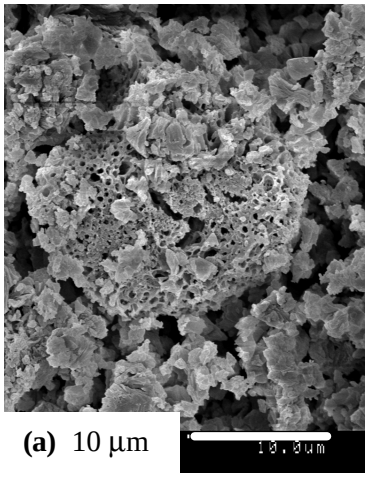


(a) 50 μm **(b)** 30 μm

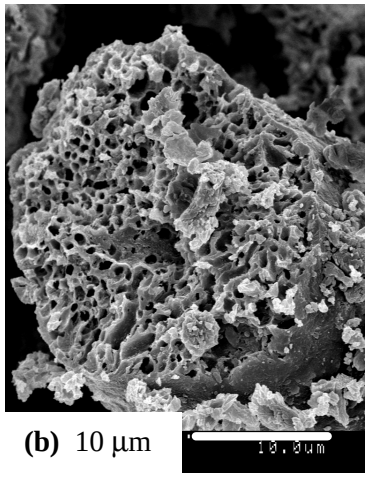
Figure 3. PMMA beads foamed using $SCCO_2$ as the porogen. Foaming media: CO_2 at 318 K and 20 MPa (20 h). Depressurization rate: 4 MPa sec^{-1}.
(a) PMMA.
(b) PMMA/TMA.

Polymer beads where also impregnated with cholesterol using the standard method of SCF-impregnation described in the literature [5,6]. In both polymers, PMMA and PMMA/TMA, impregnation values in the order of 5-10 wt% were easily reached. After depressurization, cholesterol remained impregnated in the polymeric matrix, even in those circumstances where the porosity networking was open to the exterior. Hence, a simultaneous impregnation/foaming procedure can be carried out.

To study the foaming process in the PLA powdered samples, the depressurization lasting time was varied from 1 h to 5 sec. SEM micrographs of the obtained samples are shown in Figure 4. When the pressure was released within 5 sec, samples with more percentage of porosity than those obtained by slow depressurization were observed. Moreover, larger pores were obtained in the former case.



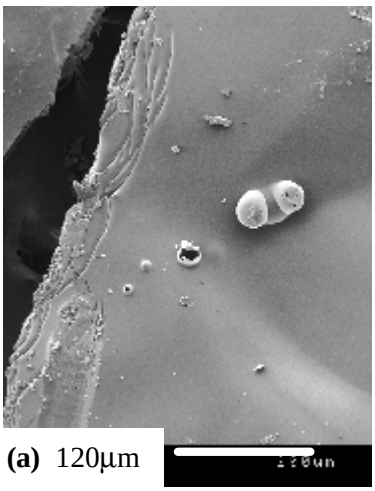
(a) 10 μm



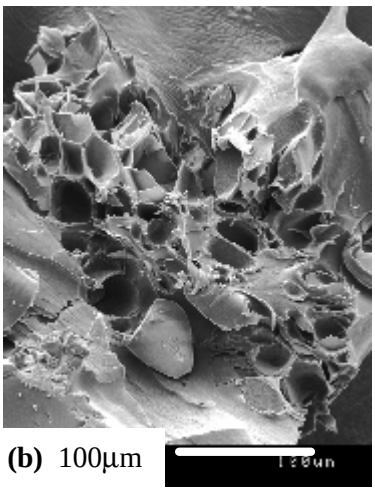
(b) 10 μm

Figure 4. PLA samples foamed using $SCCO_2$ as the porogen. Foaming media: CO_2 at 308 K and 20 MPa (20 h). Depressurization rate:
(a) 5 MPa sec^{-1}.
(b) 0.33 MPa min^{-1}.

In the reactive process, where PMMA was synthesized in a $SCCO_2$ medium, a polymer film instead of beads was obtained, since no surfactant was used. To produce foams, pressure was released relatively quickly, from 25 to 0 MPa within 2 min. SEM micrographs of the polymeric films evidenced the formation of a nonporous and denser outer skin (Fig. 5a). After fracturing the film, the cross-section displayed an internal macroporous network or microcellular foamed region (Fig, 5b).



(a) 120μm



(b) 100μm

Figure 5. PMMA film synthetized and foamed in a $SCCO_2$ medium: 338 K and 25 MPa (20 h). Depressurization rate: 3 MPa min^{-1}.
(a) Outer skin.
(b) Internal pore networking.

This particular process of foaming produced a film cross-section not unlike natural bone with respect to the distribution of porosity, although pore size should be still adjusted. To prepare optimal polymer nanocomposite foams for bone implant, the mechanical and surface properties should be improved, fi, by adding an inorganic filler. One of the most highly compatible inorganic fillers is HAP, the main inorganic component of bone, specifically nanometric HAP. The mixing of nanofillers and polymers is difficult to achieve due to the large surface energy of nanometric particles. The depression of glass transition temperature of polymers in presence of $SCCO_2$ is expected to facilitate the mixing and distribution of nanometric fillers throughout the substrate [7].

Preliminary work has shown that conventionally prepared nano-HAP particles can be converted into carbonated apatite, that closely matches the chemical composition of bone mineral, by using a SCF-route. Analysis of the HAP *a* and *c* cell-parameters, obtained from the XRD patterns of the as-synthetized and $SCCO_2$ processed samples, showed an increase in

the *c*-axis and a decrease in the *a*-axis values, after the SC-treatment. This behaviour is indicative of a type-B carbonate substitution, where PO_4 groups are replaced by CO_3 groups.

The main body of experiments of this work where designed on the basis of the ability of $SCCO_2$ to reversibly swell a polymer and promote diffusion of large molecules into this swollen matrix and the ability to produce controlled foam structures resulting in microenvironments that promote cell proliferation and implant integration. Actually, foamed polymers are very much demanded in the area of biotechnology for tissue engineering.

The final objective of the ongoing research is to obtain a material similar to bone to be used as an implant be means of a simultaneous $SCCO_2$ plasticization-mixing-impregnation-foaming method. In this article, some of the individual steps have been analyzed. All of them can be carried out in a similar $SCCO_2$ environment at comparable pressures and temperatures. Evidently, the concurrent occurrence of the different steps in the final process will induce differences in the obtained composite material. For instance, adding a small amount of nanoparticles to the polymer before foaming can greatly alter the foaming process, since nanoparticles will induce heterogeneous pore nucleation and grow. To solve some of the problems related to incompatibility, further work will be related to the incorporation of enzymes and proteins into the polymeric matrix to improve adhesion and incorporation of these materials in the body.

CONCLUSIONS

SC-CO_2 can produce a variety of foamed structures with varying pore size and distribution. PMMA beads without continuous outer skin have been prepared. Preliminary results suggest that the degree of porosity, the average pore size and the surface area in PMMA and PLA can be tuned over a wide range by varying the CO_2 density and the depressuration rate. An initial demonstration of the potential for using CO_2 to make carbonated nanoapatite, adequate for composite implants, has also been provided. A further challenge in producing nanocomposites is to reach an intimate mixing avoiding nanoparticle agglomeration, to generate a controlled porosity that promotes cell infiltration and influences polymer degradation and bioactive agent release (antibiotics or grow factors).

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