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Review

Supercritical fluids processing of polymers for pharmaceutical and medical applications

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ABSTRACT

A critical analysis is presented of the supercritical fluids based technologies that have been proposed in polymer processing for pharmaceutical and medical applications. The formation of polymer–drug microparticles and microspheres, the production of simple or loaded membranes and the formation of temporary scaffolds are reviewed and the future trends in these areas are analyzed.

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Contents

1. Introduction

One of the main reasons for polymer processing in pharmaceutical field is the aim to prepare controlled release formulations. This kind of pharmaceutical preparations are as a rule the result of a combination between polymers and drugs to obtain:

1. Fast release for drugs with low water solubilities.
2. Prolonged-delayed release for drugs with high water solubilities.
3. Protection of the active principle.
4. Minimization of haematic concentration peaks avoiding side-effects.
5. Better patient compliance.

These results can be obtained producing co-precipitated particulate systems of micrometric or nanometric diameter (microspheres, nanospheres) or by entrapping/dissoluting the drug into porous polymeric media (membranes).

The other major field that involves polymers and the health is their use in medical devices aimed at substituting/surrogating compromised functions of the human body (membranes, stents, scaffolds). Tissue engineering originates from reconstructive

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surgery where direct transplantation of donor tissue is practiced to repair the function of damaged tissue. Many difficulties arise with direct transplantation due to insufficient donor organs, rejection of the donor organ and pathogens transmission. An autogenic tissue engineering transplant (using patient's own cells) would address most limitations of direct transplantation and avoid difficulties concerning rejection and pathogen transmission. Additionally, there would be no dependency on donors. Therefore, constructing a tissue-engineered replacement in vitro can be an excellent alternative to direct transplantation of donor organs [1,2].

Supercritical fluid based technology has been largely proposed to produce materials with nanostructural properties [3]. In some cases polymers and biopolymers targeted for pharmaceutical and medical applications have been considered. The present paper analyses these latter processes, the results obtained and the perspectives of the most promising techniques.

2. Composite polymer microparticles

2.1. Supercritical antisolvent (SAS)

SAS is the most popular supercritical antisolvent precipitation process and has been used under various acronyms, mainly depending on the kind of injection system used. The injector is designed to produce liquid jet break-up and the formation of small droplets to produce a large mass transfer surface between the liquid and the gaseous phase. Several injector configurations have been proposed and patented in the literature [4–7]. In the solution enhanced dispersion by supercritical fluids (SEDS), SC-CO_2 and solvent are mixed in a tube-in-tube injector [8], in the aerosol solvent extraction system (ASES) [9] and in the precipitation by compressed antisolvent (PCA), the solution is sprayed from an injector.

The formation of composite microparticles by SAS has been recently reviewed [10–12]; however, the general observation is that the results obtained by SAS in the formation of composite microparticles are limited. Indeed, SAS precipitation can produce several morphologies: crystals, nanoparticles and microparticles [12–14]. Crystals co-precipitation with a polymer has never been reported; nanoparticles precipitation does not give co-precipitates due to the gas-to-particles mechanism that governs the process [12]. Therefore, only in the case of SAS precipitation in form of microparticles, generated by the solvent elimination from liquid droplets, it is reasonable to imagine that polymer–drug composite particles are formed. At present, only PLLA–drug microparticles [12,15–18] have been consistently reported in the literature. It has to be underlined that none of the above reported references proposed a systematic investigation on drug encapsulation efficiency and how this results can be influenced by the SAS process parameters used.

2.2. Rapid expansion of supercritical solutions (RESS)

The main limitation in the use of RESS [19] is that the compounds to be micronized have to be soluble in SC-CO_2 at the pre-expansion conditions; there are not many pharmaceutical compounds with these characteristics. Also many polymers show very limited solubilities in SC-CO_2 [20].

Mishima et al. [21] tried to overcome these limitations proposing a process called RESS-N (rapid expansion of supercritical solutions with a nonsolvent), in which the protein is insoluble and the polymer has to be soluble in a mixture SC-CO_2–solvent. The presence of the solvent in SC-CO_2 can improve the solubility of the polymer and avoids the swelling and the coalescence of the particles. During RESS-N the protein is suspended in the SC-CO_2–solvent solution and can be covered by the polymer solubilised in it, when the suspension is decompressed. The co-solvent is removed by CO_2 during the decompression and microcapsules are formed. The authors state that the best co-solvent is ethanol and the best results in term of microparticle morphology and loading have been obtained using lipase and lysozyme coated by PEG6000. Microspheres diameter ranged between 10 and 60 μm.

Using the same process, Matsuyama et al. [22] also reported the production of microcapsules of several pharmaceutically accepted polymers and pharmaceutical compounds. A question is open about the ability of the polymer to cover the suspended particles without producing particles that does not contain any drug or contain multiple particles of the pharmaceutical compound.

2.3. Particles from gas saturated solution (PGSS)

Another popular supercritical assisted precipitation process is PGSS [23,24]. To produce composite microparticles, this process requires the formation of a suspension of drug microparticles inside the selected polymer. Indeed, when the polymer melts due to heating and reduction of the glass transition temperature induced by SC-CO_2, a viscous suspension is formed that can be subsequently atomized forming composite microparticles containing the suspended solid drug on a random basis. The attempt to melt directly the drug in PGSS has failed due to the fact that drugs, as a rule, decompose before liquefaction. Even when the experiments were conducted at temperatures below the drug melting point, decomposition was not avoided. For example, mixtures of 20% nifedipine and 80% PEG 4000 (1:4) were micronized by PGSS [25] to obtain co-precipitates and the experiments were carried out at pre-expansion pressures between 120 and 190 bar and temperatures between 50 and 70 °C, below the drug melting point (172 °C). Fine powdered co-precipitates were obtained, but DSC analyses showed nifedipine degradation in the micronized product.

2.4. CO_2-assisted nebulization with a bubble-dryer (CAN-BD) and supercritical assisted atomization (SAA)

Two other atomization processes have also been frequently proposed in the literature CAN-BD [26–28] and SAA [29]. They use SC-CO_2 to improve the atomization process. The first process instabilizes the liquid jet at the exit of a capillary producing an intercalation of liquid droplets and SC-CO_2 bubbles into the capillary; the result is a large improvement of the atomization process with the production of smaller droplets. In the second process, large quantities of SC-CO_2 are solubilised in the liquid solution before atomization. The result is a two-step atomization in which the primary droplets are broken into secondary droplets by the sudden release of SC-CO_2 from their internal. Also in this case a strong reduction of droplets diameter is obtained and consequently the particles produced are very small [29]. Both these processes are based on droplets formation and drying; therefore, in principle, if the liquid solution is formed by a polymer plus a drug, they can produce composite polymer–drug microparticles.

Sievers and co-workers used CAN-BD to produce fine particles of pharmaceuticals and other materials, by aerosolization in a low-volume mixing device (e.g., a tee or a cross). In a first work [30] CAN-BD has been used to produce composite microparticles by simultaneous mixing of two liquid solutions: a water-based and an organic solvent-based solution, with SC-CO_2. To obtain this result, the authors substituted the near-zero volume tee, that characterized this process, with a cross in which the three streams converge and then are atomized in a capillary. But, heterogeneous microparticles were obtained, formed by the coalescence of drug and carrier microparticles.

In a second work [31] attenuated measles virus vaccine has been formulated with some excipients and other compounds. The most promising results were obtained using myo-inositol in water solutions and intimately mixing it with the viral suspension, before CAN-BD processing. The composite powders retain high viral activity, for a long time and with a very small water content.

Reverchon and Antonacci [32,33] adapted the SAA process to produce composite microparticles for drug release. They studied the precipitation of ampicillin trihydrate using two different carriers: chitosan, a natural polymer, and hydroxypropyl methylcellulose (HPMC), a semi-synthetic derivative of cellulose. The drug and the two different polymers were previously separately micronized using SAA and the effect of the operating conditions was studied [33–35]. Then, the co-precipitation of the drug with the two different carriers was successfully performed: spherical particles were produced operating at 105 bar and 85 °C in the saturator, mass flow ratio between CO_2 and liquid solution $R = 1.8$ and a precipitation temperature of 100 °C for HPMC as the carrier and 95 °C for chitosan. The uniform dispersion of ampicillin trihydrate in the polymer microspheres was verified using several analytical techniques. These analyses gave complementary and concordant results: the drug remains entrapped into the polymeric matrix in the amorphous state. EDX microanalysis, that can show the spatial disposition of the compounds in a micro-sample, was used to verify that each microparticle was formed by a solid solution of drug and polymer.

Either in case of HPMC/ampicillin trihydrate [33] either in case of chitosan/ampicillin trihydrate [32] no systematic effect of polymer/drug ratio on the PSDs (particle size distributions) of co-precipitates was noted. Microparticles of the first system ranged between about 0.2 and 4 μm; microparticles of the second system ranged between 0.1 and 6.3 μm. A prolonged release was obtained for SAA co-precipitates with respect to the use of the raw drug and of physical mixtures of polymer and drug. The polymer/drug ratio revealed to be a controlling parameter for drug release. Furthermore, ampicillin trihydrate entrapped into co-precipitated microparticles using SAA technique is more stable than the raw drug.

Since SAA is based on the formation of an expanded liquid solution formed by the liquid solvent–solute–SC-CO_2, the addition of a polymer produces a quaternary system that, in some cases, can give unpredictable phase behaviour and micronization results.

2.5. Emulsion drying

Recently, it has been proposed the use of supercritical fluids for the solvent extraction from emulsions as a new technique capable to overcome some of the problems of the conventional solvent evaporation technology producing polymer microspheres with a more controlled and narrower particle size. The supercritical treatment can overcome several disadvantages of the conventional process, such as high processing temperatures and long extraction times.

Supercritical extraction of emulsions [36–38] was proposed using SC-CO_2 to eliminate the organic solvent from oil-in-water (O/W) emulsions prepared with megestrol acetate and cholesterol acetate or lipid microspheres charged with ketoprofen and indometacin. The authors reported that the processing method combines the advantages of traditional emulsion based techniques (control of particle size and surface properties) with the advantages of continuous supercritical fluid extraction such as higher product purity and shorter processing times. The primary control parameter was found to be the emulsion droplet size; therefore, precipitation of particles having different sizes can be accomplished by using different emulsion formulations and optimization of the solvent–surfactant system.

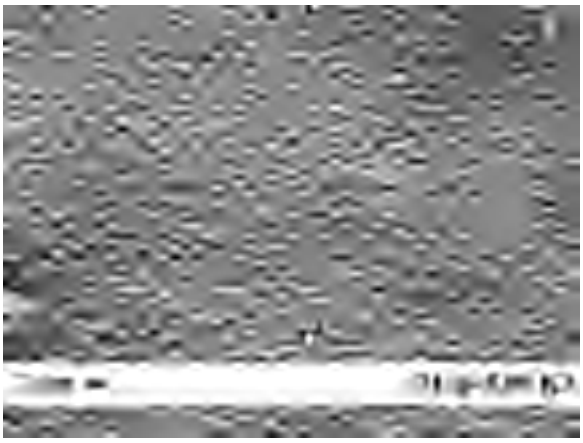

Fig. 1. SEM image of the PLGA/vancomycin microspheres obtained by SC extraction starting from W/O/W (water-in-oil-in-water) emulsion.

Perrut et al. [39] proposed the processing of an aqueous solution containing the active substance, i.e. a water-in-oil (W/O) emulsion, that is the reverse of the ones discussed in the papers described above. In this case, the emulsion was sprayed in supercritical carbon dioxide, producing drug–polymer microspheres. The proposed process is very similar to supercritical antisolvent technology. Indeed, the contact between the W/O emulsion and the supercritical solvent is obtained at the exit of the injector, where liquid drops containing the emulsion are obtained.

Della Porta and Reverchon suggested the use of an oil-in-water (O/W) emulsion prepared with ethyl acetate and water (ratio 20:80), treated by SC-CO_2 at 80 bar and 38 °C to obtain spherical PLGA/piroxicam nanostructured microspheres in 30 min of processing time [40]. The same authors also proposed a systematic comparison between the characteristics of the microspheres obtained by SC-CO_2 extraction and by conventional solvent evaporation, starting from the same emulsion [41]. Particularly, the emulsions were prepared with a different percentage of PLGA from 2.5 to 7.5% in the oily phase, and the microspheres obtained using SC-CO_2 technology, showed always a PSD narrower than the ones obtained by conventional evaporation process. An example of the microspheres produced is illustrated in the SEM image shown in Fig. 1. Other advantages of the process are a lower solvent residue and higher encapsulation efficiencies. Indeed, solvent residue after SC-CO_2 extraction is lower than 10 ppm; whereas, the conventional solvent evaporation produces microspheres with an ethyl acetate content of 500 ppm. During the evaporating process, as long as ethyl acetate is evaporated from the aqueous phase of the emulsion, a shift in the emulsion equilibrium is generated (leading to the diffusion of the organic solvent from the emulsion droplets to the continuous phase), the maximum amount of ethyl acetate that can be evaporated from water is related to the miscibility of the binary system at given operating conditions. The encapsulation efficiency of the microspheres produced by SC-CO_2 extraction was in the range between 80 and 95%; whereas, smaller encapsulation efficiency was measured in the microspheres produced by solvent evaporation. The observed result was again attributed to the very fast SC process: i.e., higher encapsulation efficiency can be expected, since the drug has less time to migrate in the continuous phase.

Della Porta and Reverchon [40] suggested the possibility of a continuous process layout where the SC-CO_2 is continuously contacted with an O/W emulsion in a column to extract the organic solvent without interacting with dispersant phase. At the bottom

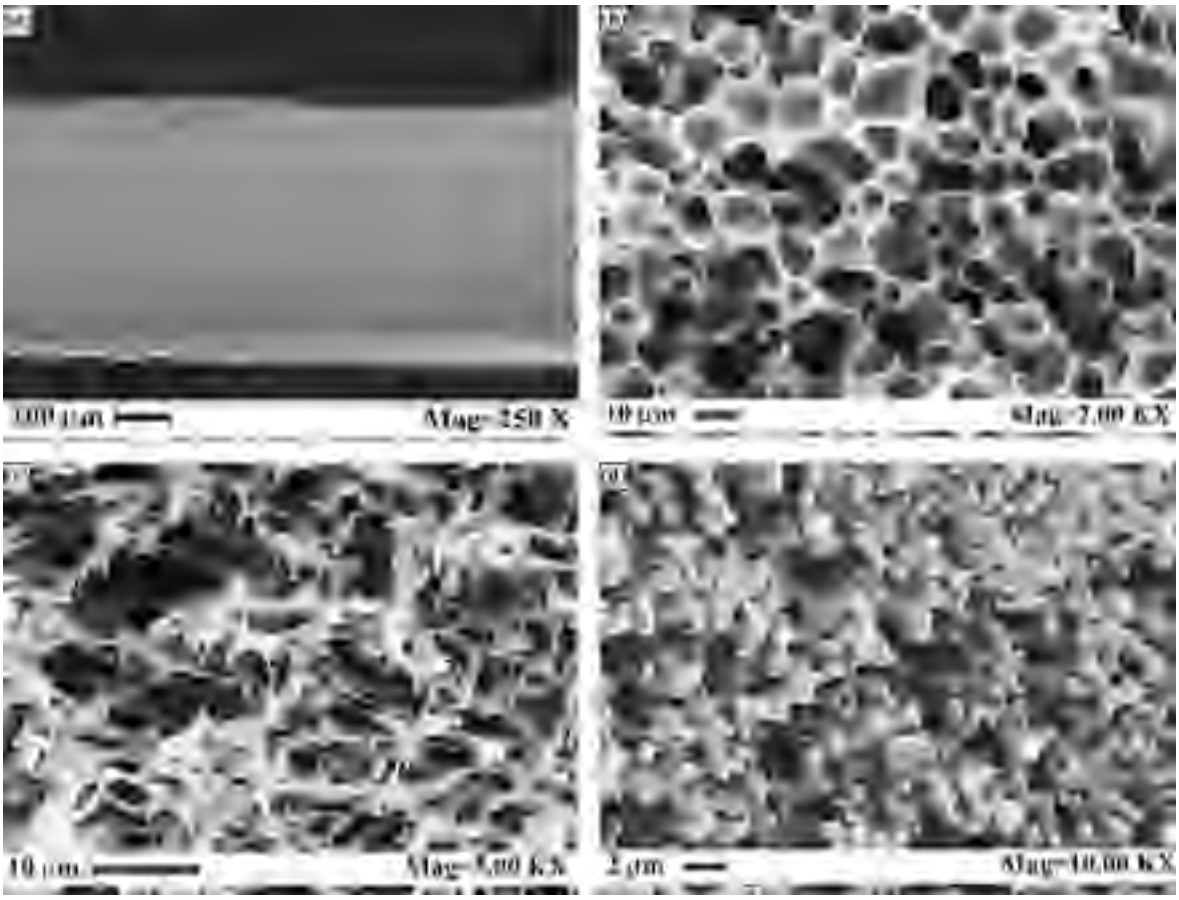

Fig. 2. Different membrane morphologies obtained with SC-IPS process: (a) dense structure (PVA), (b) cellular structure (PMMA), (c) bicontinuous structure (cellulose acetate), (d) particulate structure (cellulose acetate).

of the column, a suspension of microstructured particles can be continuously collected.

3. Polymeric and composite polymeric membranes

3.1. Supercritical phase inversion

Polymeric membranes can be used for various pharmaceutical and medical applications such as blood purification (hemodialysis, hemofiltration, hemodiafiltration), blood oxygenation (membrane oxigenators) [42]. Today, the majority of polymeric membranes are prepared by phase separation of the polymeric solution into a polymer-rich phase and a polymer-poor phase. Phase separation of polymer solutions can be induced in several ways; the two main techniques are: thermally induced phase separation (TIPS) and solvent induced phase separation (SIPS) [43]. The performance of the membranes obtained by these processes strongly depends on the morphology obtained during phase separation and subsequent solidification. SIPS process is the most used method for membranes fabrication, but suffers from various limitations. In particular, it involves the use of two solvents that must be expensively removed from the membrane with post-treatments, since residual solvents can cause problems for the use in biomedical applications. Moreover, relatively long formation times and a limited versatility characterize this process, due to the reduced possibility to modulate cell size and membrane structure once the polymer–solvent–nonsolvent system is fixed.

Recently, a process where a supercritical fluid replaces the liquid non-solvent used in SIPS has been proposed [44–60]; some biopolymers have been successfully processed [46,48–50,52–55,60]. Compared to the SIPS method, supercritical CO_2 induced phase separation (SC-IPS) process can have several advantages: (1) SC-CO_2 can form and dry the polymer membrane rapidly, without the collapse of the structure due to the absence of a liquid–vapour interface when the supercritical solvent–nonsolvent mixture is formed. Moreover, the membrane can be obtained without additional post-treatments because the polymer solvent is completely extracted. (2) It is easy to recover the liquid solvent, since it dissolves in SC-CO_2 and can be removed from gaseous CO_2 after depressurization. (3) SC-CO_2 allows to generate symmetric membranes and to modulate the membranes morphology, cells and pores size by simply changing pressure and temperature that produce different solvent powers and diffusivities.

The most relevant parameters in membranes formation by SC-IPS are polymer concentration, pressure, temperature and the kind of solvent. In some cases, other process parameters have also been investigated such as the depressurization rate and the addition of another polymer in the starting solution [54,55,57]. Depending on the process conditions and on the kind of polymer used, four main morphologies have been observed: (**2a**) dense structure, (**2b**) cellular structure, (**2c**) bicontinuous structure and (**2d**) particulate structure.

A significant example has been reported in the case of PVA membranes formation where, simply changing the SC-CO_2 solvent power, all the possible membrane morphologies reported in Fig. 2 have been obtained starting from the same polymer–solvent system [56].

To explain these results, it is possible to refer to the traditional phase inversion method using a qualitative ternary phase diagram, polymer/solvent/SC-CO_2, containing the various "composition paths". A ternary phase diagram is usually formed by a liquid–liquid (L–L) demixing gap, divided into a region of spinodal demixing, two regions of nucleation and growth located between the binodal and the spinodal curve and a gelation region. Depending on the region where the phase separation occurs, it is possible to obtain the four different morphologies:

1. *Dense structure*: the concentration of the polymer in the ternary system increases because the outflow of the solvent from the solution is faster than the inflow of the SC-CO_2; the phase inversion does not occur and the polymer molecules solidify by gelation and/or crystallization into a dense structure (Fig. 2a)
2. *Cellular structure*: the ternary solution – polymer/solvent/SC-CO_2 – becomes metastable; nucleation and growth of droplets of the polymer-lean phase occurs with solidification of the polymer-rich phase, leading to a cellular structure; in this case, both porous or dense skins can be obtained depending on the polymer/solvent/SC-CO_2 system processed (Fig. 2b).
3. *Bicontinuous structure*: the ternary solution – polymer/solvent/SC-CO_2 – becomes unstable; spinodal phase separation with the subsequent solidification of the polymer-rich phase takes place, leading to the formation of a bicontinuous structure; in this case, porous skins are usually obtained (Fig. 2c).
4. *Particulate structure*: in this case nucleation and growth of droplets of the polymer-rich phase is obtained, followed by solidification of the polymer-rich phase; a beads-like (particulate) structure is obtained (Fig. 2d).

In the case of SC-IPS process, the "composition paths" can be strongly influenced by the process parameters. Indeed, when the SC-CO_2 solvent power is limited, the outflow of the solvent from the solution is favoured; the path does not "enter" in the miscibility gap but moves towards the pure polymer vertex leading to the direct accumulation of polymer, i.e. dense structure formation (1). Increasing SC-CO_2 solvent power, the process becomes faster and the outflow of the solvent decreases; in this way, the pathways shift towards the lower part of the diagram "entering": inside the upper demixing gap (between spinodal and binodal curves) leading to a cellular structure (2); or, in the central region of demixing gap leading to bicontinuous structures (3); or, towards the pure antisolvent vertex leading to microparticles formation (4). These results confirm the high versatility of the SC-CO_2 assisted process.

A further pharmaceutical application of polymeric membranes is the production of drug controlled release devices, loading a drug in the membrane. The release of a drug in a sustained release formulation is controlled by different mass transfer mechanisms such as diffusion, erosion, swelling or osmosis, depending on membrane formation process, on material properties (composition, porosity, roughness, wettability and water uptake) and on drug properties, such as solubility and molecular weight [61].

Loaded PMMA membranes prepared by SC-IPS have been reported by Reverchon et al. [62]. PMMA porous membranes have been prepared and loaded with an antibiotic, amoxycillin, using two different techniques: dissolving it in the same organic solvent used to solubilize the polymer or suspending the drug in the organic solution formed by polymer and solvent. The porous membranes were produced at various drug loading and characterized by SEM, to study the morphology and cells size, and by DSC to analyze the interaction of drug–polymer in the structure.

The presence of the suspended drug does not interfere with the PMMA membrane structure morphology (i.e., a cellular structure is obtained as in the case of pure PMMA membranes as shown in Fig. 2b). When the drug is initially dispersed, it remains in this form during the membrane formation process, since the SC-IPS is an extremely fast process.

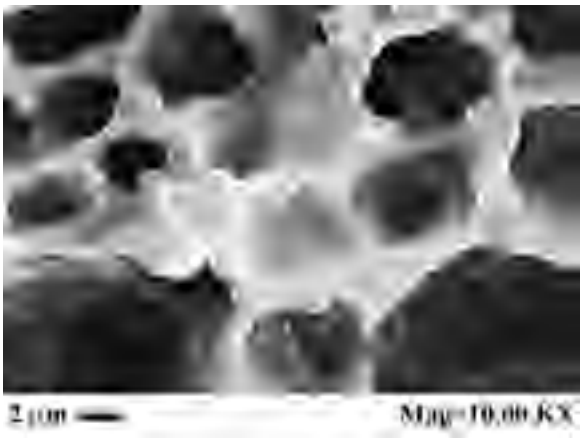

Fig. 3. High magnification of a PMMA structure prepared using acetone, containing 30 wt/wt% of amoxycillin (20 MPa, 45 °C and 80% (wt/wt) acetone); cells wall roughness due to drug incorporation is evidenced.

Cell walls present a distributed irregularity (roughness) that was not observed in pure PMMA structures (Fig. 3). This morphology is due to the presence of the drug; i.e., amoxycillin is encapsulated inside the polymeric structure. In the case of drug dissolved in the same solvent of the polymer, cell surface is smooth for all drug concentrations tested.

Some drug release experiments were also performed to verify the efficiency of SC-CO_2 assisted encapsulation process and to study the effect of the collocation of drug in the structure on the release kinetics. Untreated amoxycillin dissolves completely in 10 min; whereas, it was observed an amoxycillin prolonged release of 20 h in the case of the dissolved drug PMMA membranes. No burst effect was observed; i.e., no initial fast release of the drug has been verified [62]. It means that no drug is present in the outer layer of the structure as it frequently happens using traditional loading methods.

3.2. Emulsion templating

Ryoo et al. [63,64] focused some studies on the possibility to use SC-CO_2 as the continuous phase of an emulsion, producing water-in-CO_2 emulsions (W/C) or, alternatively, as a dispersed phase of an emulsion, producing CO_2-in-water emulsions (C/W) [65]. Several surfactants were also studied and optimized by the authors to stabilize these emulsions. The C/W or W/C reverse micelles have the advantage that no organic solvents are used and the presence of SC-CO_2 adds further flexibility to the process since the variation of density with pressure and temperature allows to use it as a tunable medium for reaction and separation processes.

Based on these results, Cooper [66] developed a method for templating a C/W emulsion to generate highly porous materials in absence of organic solvents [67,68]. Indeed, providing that C/W emulsions are sufficiently stable, due to the use of a good surfactant system such as, perfluoropolyether and poly-vinyl alcohol, it is possible to generate poly-acrylamide low density material (0.1 g/cm^3) with a relatively large pore volume from water soluble vinyl monomers such as acrylamide and hydroxyethyl acrylate. These authors also reported that increasing the volume fraction of the CO_2 internal phase increased porosity and increasing the surfactant concentration led to open interconnected structures. The results obtained are very interesting and reveal that using emul-

sion templating, a large variety of porous hydrophilic materials can be produced by reaction or induced phase separation of concentrated C/W emulsions e.g. using sol–gel chemistry, or free radical polymerisation. Indeed, the removal of the internal phase (formed by CO_2) yields the porous product; whereas, the conventional methods require large amounts of water immiscible organic phases as the internal phase (usually >75%), which can be difficult to remove after the reaction.

A variation of emulsion templating technique has been proposed by Partap et al. [69]; they developed a new method to produce porous alginate hydrogels by combining emulsion templating with an internal gelation reaction. Highly porous alginate hydrogels with a narrow range of macropore sizes were made using the so-called reactive emulsion templating (RET) which utilizes a C/W emulsion to template the pores. In the RET process, CO_2 plays a dual role: as a reagent increasing the acidity of the aqueous phase to initiate gelation of alginate and as a templating oil phase agent. The produced hydrogels showed an open, well-interconnected pore network with a narrow pore size distribution that may potentially be suitable for tissue engineering applications.

4. Temporary scaffolds

One of the major research themes of polymer processing in medical field is scaffold fabrication; a scaffold is a 3D porous construct which serves as temporary support for cells to grow into a new tissue, before it is transplanted back to the host tissue. Even if the substitution of different biological materials requires different scaffold characteristics, they share a series of common features that have to be simultaneously obtained [2,70]: (1) a high regular and reproducible 3D structure (macrostructure); (2) a porosity exceeding 90% and an open pore geometry that allows cells growth and reorganization; (3) a suitable cell size depending on the specific tissue to be replaced; (4) a high internal surface areas and a proper nanostructural surface characteristics that allow cell adhesion, proliferation and differentiation; (5) mechanical properties to maintain the pre-designed tissue structure; (6) biodegradability, biocompatibility and a proper degradation rate to match the rate of the neo-tissue formation.

Several techniques have been proposed for scaffolds fabrication that include: fiber bonding, solvent casting, particulate leaching, melt moulding, solid free form fabrication, gas-foaming and freeze drying combined with particulate leaching [70,71]. But, they suffer various limitations; particularly, it is very difficult to obtain the coexistence of the macro and microstructural characteristics that have been previously described.

Until now the use of SC-CO_2 in this field, has been limited to gas-foaming techniques in which it is used as a porogen [72,73,73–82]. The process is solventless and very efficient in producing the porous structure; but generally closed-cells structures are generated during this process. Indeed, though Mooney et al. [72] claimed that open porous structures could be obtained using this process; the same author [73] in a subsequent work adopted a CO_2-foaming plus solid porogen technique (i.e., foaming/particulate leaching technique) to overcome the limited cell connectivity of the scaffolds. However, Barry et al. [75,80] highlighted the limitations of the leaching process used by Mooney and co-workers [73] such as long manufacturing time and difficulty in porogen elimination. Barry et al. prepared methacrylate scaffolds by SC-CO_2 foaming and claimed that the degree of porosity and interconnectivity of scaffolds can be controlled simply by modifying the depressurization rate of the process: scaffolds with a porosity of about 89% and with high connectivity (74% open pores) have been produced [75,80] according to these authors. This result is somewhat surprising since, as a rule, foaming processes tend to produce a low cell interconnectivity. An attempt to control and improve the interconnectivity of the cells by post-processing the scaffolds has been proposed by Wang et al. [82]. They produced foams of PLA (using sub-critical CO_2) and exposed them to pulsed ultrasound at a frequency of 20 kHz and an average power input of 100 W. According to the authors, this post-processing not only slightly increased the cells size, but also improved their connectivity as a result of cell wall rupture. On the other hand, as the scaffolds porosity increases, the mechanical strength decreases precluding the use of scaffolds in applications that require high mechanical resistances. For this reason, Barry et al. [78] investigated the foaming of blends of THFMA with styrene–isoprene–styrene copolymer elastomer for soft prosthetic applications. According to these authors, the degree of porosity and interconnectivity of the cells could be tuned modifying the blend composition and the processing temperature. Similarly, Mathieu et al. [81] have shown that the morphology of the foams can be controlled to mimic the bone structure. Bone from different sites around the body is anisotropic, both morphologically and mechanically. They found that similar anisotropic structures can be formed by controlling the depressurization rate of the foamed PLLA and the density of the gas: rapid depressurization locked in large numbers of spherical cells, whereas a slower depressurization enabled cell elongation. However, in the foaming process, the rough nanofibrous internal structure that should mime the natural extra-cellular matrix necessary to obtain a good cell adhesion and growth is completely missing.

SC-IPS process has also been proposed to generate scaffolds of PLLA by Tsivintzelis et al. [83]. The uniform cross-sections and the cellular pores of the final samples indicated the occurrence of a liquid–liquid (L–L) demixing process, followed by crystallization of the polymer-rich phase, as the dominant mechanism of the phase separation and pore production. According to these authors, it is possible to control the scaffolds morphology changing the operative conditions; indeed, the average pore size decreased with the increase of CO_2 density either by increasing the pressure or by decreasing the temperature, whereas the average pore diameter decreased with the increase of the initial polymer concentration.

Subsequently, the same authors loaded PLLA scaffolds with montmorillonite with the aim of improving the mechanical and physical properties of the polymeric matrix [84]. The analysis of the results showed that the introduction of a small amount of the organically modified mineral clay has a major effect on the final porous structure. All the nanocomposite materials exhibited more uniform cellular structures with large pores than the pure polymer. However, the structures obtained using this approach suffer various limitations. Indeed, it is very difficult to obtain complex 3D structures (i.e., usually films or hollow fiber membranes are generated) and the presence of the rough nanofibrous internal structure that should mime the natural extra-cellular matrix necessary to obtain a good cell adhesion and growth (smooth cell walls are usually obtained).

To fulfil the necessity of producing interconnected microcells and a nanometric substructure, a new supercritical fluid assisted technique for the formation of 3D PLLA scaffolds has been proposed [85]. It consists of three sub-processes: the formation of a polymeric gel loaded with a solid porogen, the drying of the gel using SC-CO_2, the washing with water to eliminate the porogen.

When PLLA gel drying is performed by supercritical CO_2, the supercritical mixture formed during the process (solvent + CO_2) has no surface tension and can be easily eliminated in a single step by the continuous flow of SC-CO_2 in the drying vessel. The major problem in gel drying is the possibility of gel collapse. In this case, the absence of surface tension avoids this problem preserving the nanoporous structure. On the other side, large interconnected

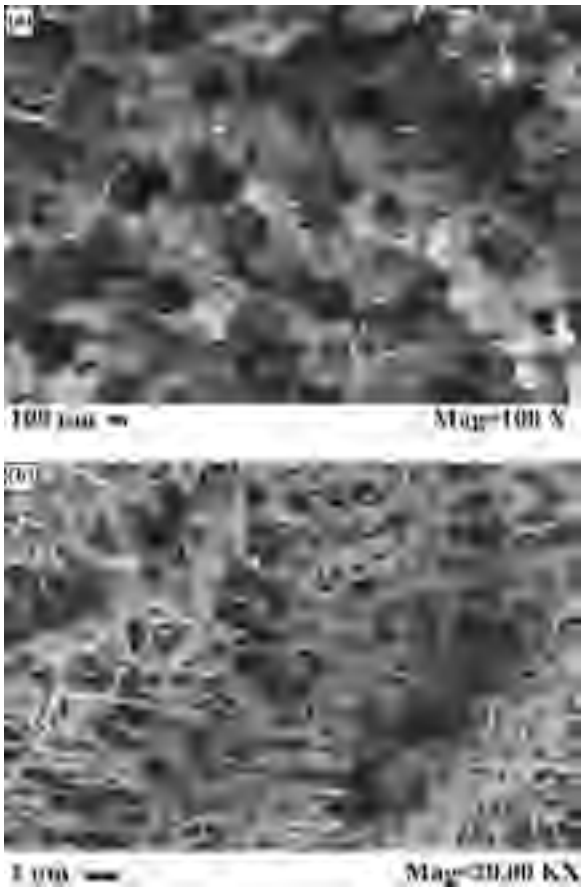

Fig. 4. SEM images of the same part of a PLLA scaffold obtained at different magnifications: (a) image of cells produced by porogen inclusion; (b) enlargement of part of a cell (i.e., nanofibrous sub-structure).

cavities necessary to mime the tissue to be replaced are completely missing. To overcome this limitation, the authors proposed a hybridization of the supercritical drying process with the particulate leaching. Indeed, it is possible to produce large cells inside a polymeric matrix using a porogen, an insoluble compound, that is introduced into the polymeric solution before the gelation. They successfully processed large and complex 3D gel structures (i.e., bone-shaped gels 10 cm long), confirming the possibility of producing scaffolds with a specific geometry without size limitations and characterized by porosity of about 95%. PLLA scaffolds produced using this process have the following characteristics: (1) adequate nanostructure; this property is conferred to the scaffold by the original fibrous nanometric substructure that is characteristic of the polymeric gel; (2) accurate reproduction of the shape and 3D structure of the tissue to be substituted; (3) controlled and large porosity (>90%); (4) very large connectivity at micronic and nanometric levels; (5) very short processing time; (6) highly reduced solvent residues.

The interconnection among the cells increasing the pore connection was also optimized by pressurizing at 10 bar the suspension liquid solution + porogen. SEM images are shown in Fig. 4. It is possible to observe the cellular structure (induced by porogen inclusion) and the nanofibrous substructure (due to polymeric gel) characterizing not only the polymeric network, but also the cells wall. In particular, fibers ranging between 50 and 500 nm add further porosity and interconnectivity and produce the walls "roughness" that should be the key factor for proteins and cellular adhesion and growth.

Fig. 4b confirms that the presence of porogen particles during the gelation process did not influence the nanoporous structure formation.

5. Conclusions and future trends

The SC-CO_2 assisted processes illustrated in this work substantiate the premise that these processes are fast and capable of producing composite microparticles or porous polymeric structures for pharmaceutical and medical applications. The morphology can be tuned through the proper selection of the process parameters.

Composite microparticles have already been produced on a pilot scale by SAA at University of Salerno and we expect their further application.

SC-emulsion processing for the production of drug–polymer microspheres, microcapsules or microporous templates are now under evaluation. Efficient drug encapsulation by supercritical extraction of emulsion and well-defined microporous structures by emulsion templating are two emerging fields of the future [40,66].

In the immediate future, we expect that SC-IPS process will be also tested for the formation of other kind of membranes such as capillary membranes for dialysis application (a continuous version of SC-IPS has been patented [86]) and polymeric blends to combine the characteristics of different polymers. An example of polysulfone/polycaprolactone blends has been recently proposed by Temtem et al. [54].

The optimization of polymeric scaffolds characteristics is also very relevant. For example, the possibility of uniformly distributing nanosized substances (i.e., hydroxyapatite nanoparticles) inside the scaffolds structure, with the aim of obtaining an increase of mechanical properties and a better "imitation" of the human bone, has been recently proposed [87].

Another interesting SC-CO_2 assisted process, that is emerging, is supercritical (SC) electrospinning for the formation of wiring structures. Electrospinning of polymeric fibers is an area receiving increasing attention and small diameter fibers ($\ll 1\ \mu m$) have been produced using this method from a wide variety of polymers. SC-electrospinning can take advantage of the solubility of the supercritical solvent in the polymers to reduce the polymer viscosity for fiber "spinning". The first attempts at the feasibility of this process have been proposed [88,89].

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