LOADED POLYMERIC MEMBRANES FORMATION USING A SUPERCRITICAL FLUIDS ASSISTED PROCESS

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ABSTRACT

Loaded polymeric membranes have been prepared using a supercritical fluid based phase inversion process in which CO_2 acts as the nonsolvent. We preliminary tested the possibility of producing PMMA membranes using the supercritical fluid assisted phase inversion process and of modulating their properties changing the process parameters: polymer concentration, temperature, pressure and liquid solvent. Then, we prepared polymeric membranes loaded with model drugs: amoxicillin and cefuroxime. To obtain this result, we used two process alternatives: the dissolution of the drug in the organic solvent used to solubilize the polymer or the suspension of the drug in the organic solution formed by polymer and solvent. The loaded membranes, produced at various drug loadings, were characterized by SEM, to study the morphology and cells size, by DSC to analyze drug-polymer interactions in the membrane. Drug release rate analysis were performed to observe the different release behaviors.

INTRODUCTION.

Poly(methyl methacrylate) (PMMA) is a polymer largely used in biomedical applications. It has a good degree of compatibility with human tissues [1-3]. A relevant biomedical application of PMMA is represented by the production of porous structures (membranes) to be used as controlled release devices for pharmaceutical products [4,5]

Many methods for fabrication of tissue-engineered scaffolds and drug delivery devices from polymers involve the use of organic solvents [6, 7]. However, devices fabricated using organic solvents are commonly dried under high vacuum for 12 to 48 h. This method can lead to the collapse of pores in the structure. Moreover, some polymer formulations may retain very high levels of solvent. Residual solvents in implantable devices are subject to regulatory restrictions.

Supercritical CO_2 (SC-CO_2) phase inversion offers an attractive and alternative process to obtain solvent free structures. Usually, the phase inversion of polymeric solutions is obtained by liquid nonsolvent addition. The polymer solution is immersed into a coagulation bath filled with a non-solvent: the solvent diffuses out the casting film while the coagulant (non-solvent) diffuses into it. The contact between the solvent and the non-solvent causes the phase transition and polymer precipitation generating membranes. The substitution of the liquid non-solvent with SC-CO_2 can produce several advantages: SC-CO_2 can dry the membrane rapidly without the collapse of the structure due to the absence of a liquid-liquid interface, the process does not require additional post-treatments and it is easy to recover the organic solvent. Therefore, some works on membrane generation using SC-CO_2 have been proposed in the literature [8-10]. Our research group studied the formation of cellulose acetate membranes from acetone [8], polysulfone membranes from N-methylpyrrolidone (NMP) and chloroform [9] and PMMA membranes from DMSO, acetone and THF [10]. We demonstrated that it is possible to modulate cell and pore size changing the process parameters. Moreover, in some cases, we obtained different membrane morphologies: cellular structure, binodal structure and microparticles.

Since SC-CO_2 phase inversion process has several advantages with respect to traditional phase inversion process, in this work we have tested this method to produce loaded PMMA membranes. The effect of different organic solvents and of the drug concentration on membrane morphology and cell size has been analyzed. Preliminary release rate experiments have also been performed.

MATERIALS AND METHOD

Materials

Poly-methyl-methacrylate (PMMA, molecular weight 120,000), acetone (purity 99.8%), dimethylsulfoxide (DMSO, purity 99.5%) and amoxicillin (purity 99.6%) were bought from Sigma-Aldrich; CO_2 (purity 99%) was purchased from S.O.N. (Società Ossigeno Napoli, Italy), cefuroxime was, kindly supplied by Farmabios (Pavia, Italy). All materials were processed as received.

Membrane preparation and characterization

Membranes were prepared in a laboratory apparatus equipped with a 316 stainless steel high-pressure vessel with an internal volume of 80 mL. PMMA was dissolved in the solvent; drug was loaded into the PMMA solutions by dispersing it at various polymer/drug weights ratio. Membrane casting process is similar to the traditional procedure. The solution (or dispersion) was placed in a membrane formation cell (steel caps with a diameter of 2.5 cm and height of 300 μm) spreading it with a glass stick to control the thickness of the film. The cell was rapidly put inside the membrane preparation vessel to avoid the evaporation of the solvent. The vessel was closed and filled from the bottom with SC-CO_2, up to the desired pressure using a high- pressure pump (Milton Roy–Milroyal B, France). We operated in batch mode for 45 min; then, a micrometric valve was opened and the operation was performed in continuous mode for 45 min; i.e., with a constant CO_2 flow rate set at 1.5 kg/h and with a constant pressure and temperature. Then, the vessel was slowly depressurized for 30 min.

PMMA membranes were examined by cryofracturing them with a microtome (Bio-optica S.p.A, Italy, Mod. Microm HM 550 OMVP) by sputter coating the sample with gold and viewing it by scanning electron microscope (SEM) (mod. LEO 420, Assing, Italy) to determine cells size and membrane structure. Sigma Scan Pro 5.0 software (Jandel scientific, San Rafael, CANADA) and Origin 7 software (Microcal, Northampton, USA) were used to determine the average diameter of the cells and to calculate cell distributions. We measured approximately 350 cells for each distribution.

DSC measurement of loaded PMMA membranes was carried out using a Mettler (mod. TC11, USA) differential scanning calorimeter at a heating rate of 10°C/min under nitrogen atmosphere. DSC measurements of pure components was also performed: amoxicillin has a melting temperature (T_m) of 140°C, and cefuroxime of 200°C; PMMA glass transition temperature (Tg) is 105°C.

In vitro release assays were performed to determine the kinetics of drug release from membranes. The drug loaded membrane was immersed in a glass vial containing a physiological saline solution (pH 7.2) as releasing medium (1000 mL). The sealed vial was then placed in an oven at 37°C and shaken at 200 rpm. At predetermined time intervals, a sample was filtered and the concentration of drug was assayed using a UV spectrophotometer (Varian, mod. Cary 50 Scan, Palo Alto, USA).

RESULTS AND DISCUSSION.

In a previous work we tested the possibility of producing PMMA membranes using the supercritical fluid assisted phase inversion process [10]. PMMA membranes were successful produced. Therefore based on our previous results, in this work we selected a solvent-PMMA solution at 80% w/w solvent and used acetone and DMSO as organic

solvents. A drug can be loaded in a polymer using two different techniques: dissolving it in the organic solvent used to solubilize the polymer or forming a suspension of the drug in the organic solution formed by polymer and solvent. These two strategies can give very different results in terms of membrane formation, drug release kinetics. All experiments were performed at the same membrane formation conditions; i.e. operating at 200 bar, 45°C and with a membrane thickness of 300 μm.

PMMA- amoxicillin.

Amoxicillin is an antibiotic; it has been selected as a model drug to test loaded PMMA membranes formation. It is not soluble in acetone but it is soluble in DMSO; therefore, we mixed 80% of solvent, acetone in the case of the suspension and DMSO in the case of the solution, and 20% of a mixture formed by PMMA and amoxicillin. We performed the experiments varying the percentage of drug in the mixture, from 0 to 30% w/w in PMMA, and reducing correspondently the amount of polymer to maintain the same percentage of the liquid solvent.

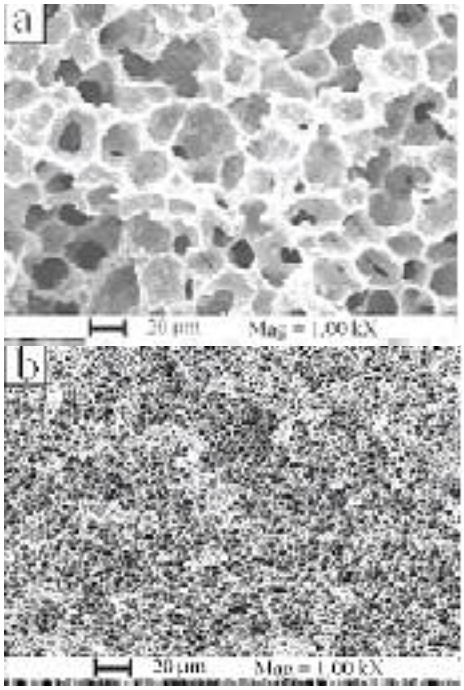

Figure 1. SEM images of PMMA membranes prepared using acetone: a) containing 30% of amoxicillin and b) pure PMMA.

A comparison between unloaded and loaded PMMA membrane is reported in figure 1 in which SEM images of PMMA membranes obtained at 0 and 30% w/w of amoxicillin are presented. The presence of the suspended drug does not interfere with the membrane morphology (i.e., a cellular structure is maintained), on the contrary it lead to a relevant increase of cells size. Probably, it depends on the fact that amoxicillin dispersed in the solution does not influence the kind of mass transport mechanism occurring during the phase separation; but the increase of drug percentage is accompanied by a decrease of the polymer percentage in the solution; therefore, the quantity of polymer-lean phase increases; moreover, the presence of the drug influences the polymer rich phase solidification acting as an "obstacle" in the organization of the solid phase. If the drug is initially dispersed and remains in this form during the process, the drug it should be physically suspended in the final membrane structure. This characteristic is better evidenced in figure 2; in which amoxicillin encapsulated inside the membrane is evidenced by cells surface roughness. Using DMSO as solvent, the membrane obtained show an uniform yellow color that is characteristic of amoxicillin; whereas, in the experiments with the amoxicillin suspension membrane color remained white. Some examples of these membranes are reported in figure 3 in which SEM images of membranes containing 0 and 30% w/w amoxicillin in PMMA are reported. In this case, an increase of drug percentage lead to a decrease of the mean diameter of cells. This result can be explained considering that both polymer and drug are dissolved and, increasing the drug

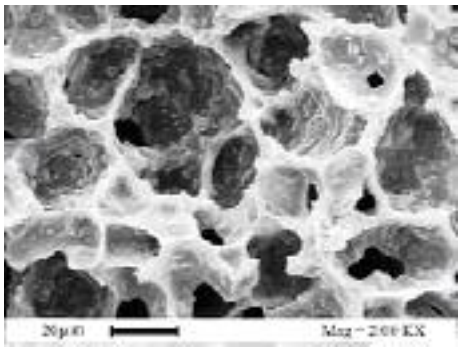

Figure 2. Higher magnification of PMMA membrane shown in figure 1a.

percentage, decreases the amount of solvent available to polymer dissolution; as consequence we obtained the equivalent of an increase of polymer concentration and a reduction of cells size.

In this case, however, cells surfaces are smooth for all drug concentrations tested. Another important information about the state of the drug in the polymer structure can be obtained by DSC analysis. Indeed, DSC can give qualitative and quantitative information about the physicochemical status of the drug in the membrane. In figure 4, the thermograms of PMMA membranes containing 30% w/w of amoxicillin obtained from acetone and DMSO are shown. The DSC of pure amoxicillin and PMMA are also reported for comparison purposes. Trace "d" in figure 4 shows the characteristic fusion peaks of pure amoxicillin. These same peaks can be observed in the membrane containing the suspended amoxicillin (traces "c") though the peak is smaller and shifted to lower temperatures. The lowering of melting temperature and enthalpy of Amoxicillin suggests an interaction characteristic of physical mixtures [11-14]. On the contrary, in the thermograms of the membranes containing dissolved amoxicillin, (trace "b"), even at high amoxicillin percentages, the characteristic peak(s) of amoxicillin are not present.

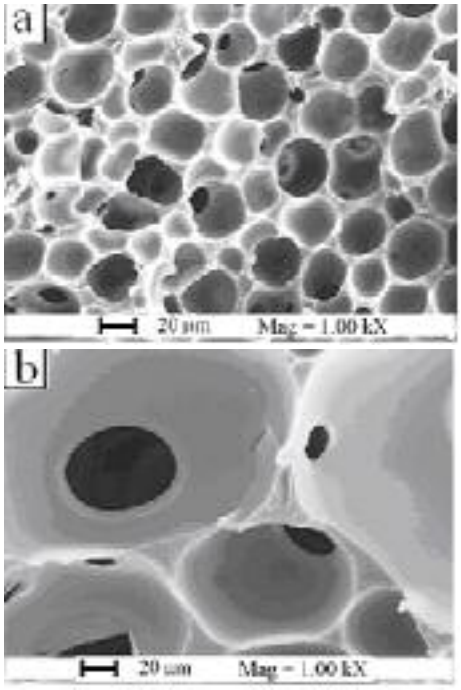

Figure 3. SEM images of PMMA membranes prepared using DMSO: a) containing 30% of amoxicillin and b) pure PMMA.

All membrane characteristics observed (color, cell surface, DSC analysis) put in evidence that the two procedures lead to totally different structures; in particular, we can suppose that, when amoxicillin is dispersed in the starting polymeric solution, polymer and drug maintained their physical characteristics in the membranes; i.e., the system behaves as a physical mixture of two components and substantially confirms the results of SEM analysis.

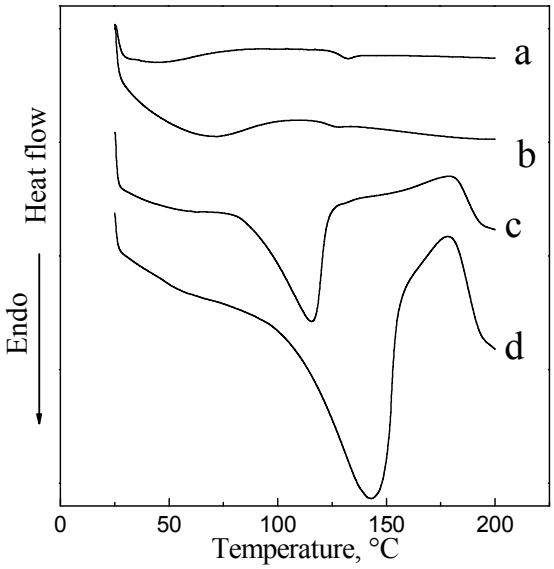

Figure 4. Thermograms of PMMA membranes: a) PMMA, b) 30% amoxicillin dissolved, c) 30% amoxicillin dispersed, d) Amoxicillin.

When amoxicillin is dissolved together with PMMA in the starting solution, it is homogenously distributed inside the polymeric matrix forming a solid solution [11]. At this point of the work, we performed some drug release experiments to verify the efficiency of SC-CO_2 encapsulation process and to study the effect of the collocation of drug in the membrane on the release kinetics. We performed experiments using the PMMA membranes containing 30 % w/w of Amoxicillin dissolved and dispersed. Figure 5 reports the release rate curves of untreated Amoxicillin and

of PMMA membrane containing dissolved and dispersed drug. Untreated Amoxicillin dissolved completely in 10 minutes. In the case of the PMMA membrane containing dissolved amoxicillin, no burst effect was observed: it means that no drug is present in the outer layer of the membrane. No initial fast release of the drug has been observed and an Amoxicillin prolonged release of 20 hours was obtained. These results agree with the previous considerations based on SEM and DSC analysis and confirm that Amoxicillin forms a solid solution with PMMA. In the case of PMMA containing dispersed amoxicillin, the drug contained in the membrane dissolves completely in three hours. Moreover, observing the initial slope of the release rate curve some considerations are possible: an initial burst release observed (from 0 to 25 min) indicates that, probably, in this case, part of the drug particles are exposed at the surface of polymer membrane. Then (from 25 to 100 min), the release rate decreases because the remaining amoxicillin particles are entrapped inside the cells of membranes.

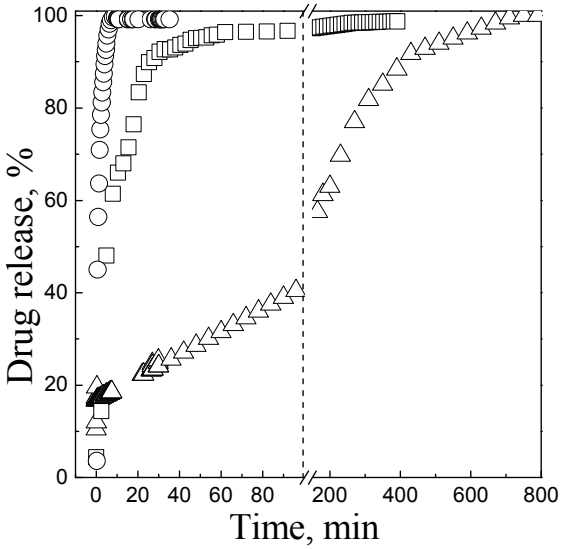

Figure 5. Release curves of: ○ untreated amoxicillin and membrane obtained starting using amoxicillin □suspension and, Δ solution.

PMMA-Acetone-cefuroxime.

Cefuroxime is not soluble in acetone; therefore, we prepared a suspension containing 80% of acetone and 20% of mixtures constituted by PMMA and amoxicillin. In this case, we performed a set of experiments varying the percentage of dispersed drug from 10 to 30% w/w PMMA. In Figure 6 examples of SEM images of membranes obtained at 10 and 30% w/w of cefuroxime in PMMA are reported. Similarly, as for the system PMMA-acetone-amoxicillin, increasing the percentage of cefuroxime, the mean diameter of cells increases from 3 to 7 μm as shows fig 7. Indeed, since cefuroxime is not soluble in acetone, increasing drug percentage, decreases the amount of polymer in the solution and the cells size increase.

DSC analysis of the various membranes is shown in figure 8. As shown in trace "e", cefuroxime has a melting temperature (T_m) of 200°C; this characteristic peak is not detectable in the case of cefuroxime loaded membranes. This result is different from the result obtained in the case of dispersion of amoxicillin and indicates that cefuroxime is probably in a metastable molecular dispersion, namely, it remains molecularly

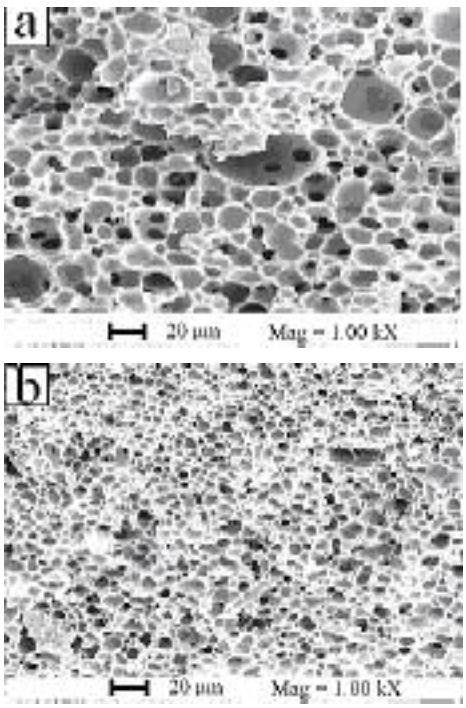

Figure 6. SEM images of PMMA membranes prepared using acetone: a) containing 30%, and b) 10% w/w of cefuroxime

dispersed in the polymer because of high viscosity of the medium and therefore crystallization is inhibited [11].

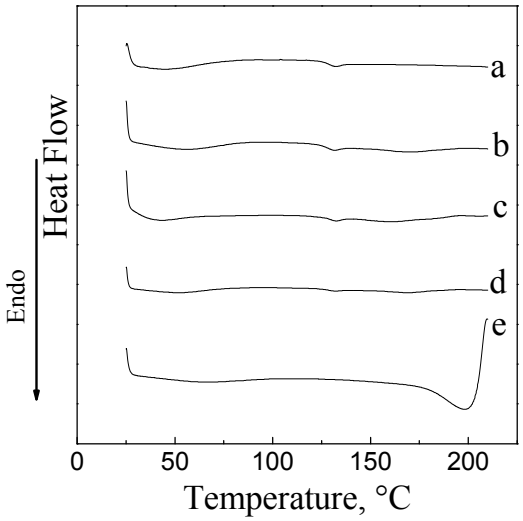

Figure 8. Thermograms of PMMA membranes, prepared using acetone, containing different percentage of cefuroxime: a) PMMA, b)10%, c) 20%, d) 30%, and e) cefuroxime .

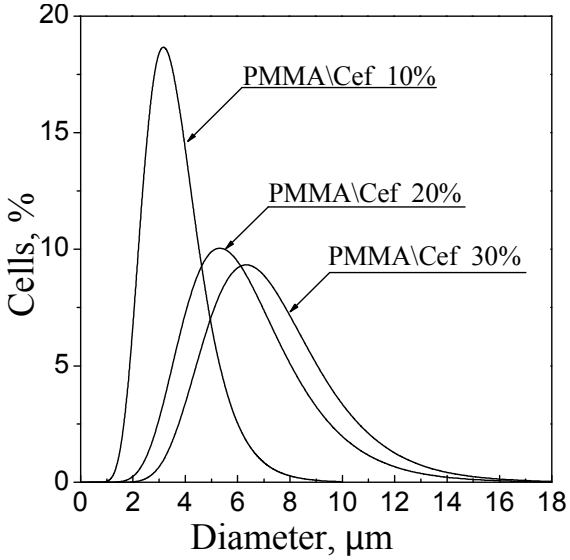

Figure 7. PMMA cell size distributions at different cefuroxime percentages (200 bar, 45°C and 80% (w/w) of acetone).

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