Optimization of HYAFF Membranes Morphology Produced by Supercritical Phase Separation for Biomedical Applications

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In this study, it was evaluated the capability to generate HYAFF membranes by phase inversion assisted by supercritical fluids. HYAFF is a biopolymer, hyaluronic acid ester, much used in the pharmaceutical and biomedical applications. Several techniques have been tested to generate structures (membranes and scaffolds) for biomedical applications; but, all of them present several limitations. For these reasons, supercritical fluids assisted processes have been implemented for biomedical applications.

Using SC-CO_2 phase inversion process, we obtained HYAFF membranes with different morphological characteristics, depending on the process conditions adopted, such as polymer starting concentrations, pressure (ranging from 90 to 200 bar) and temperature (ranging from 35 to 55°C). The behavior of membranes structure has been studied and has been related to the ternary diagram HYAFF-DMSO-SC-CO_2 analyzing the thermodynamic and kinetic aspect of the process.

1. Introduction

HYAFF is a biopolymer, hyaluronic acid ester, much used in the pharmaceutical and medical as excipient for the preparation of many drugs and as a structural element of support (scaffolding) in the care of burns therapies. This polymer has been already studied in the literature and various works have been proposed regarding its use in biomedical applications (Halbleib et al., 2003).

Several techniques have been tested to generate structures (membranes and scaffolds) for biomedical applications, such as freeze drying, rapid prototyping, wet phase inversion, foaming, evaporation, etc.; but, all of them present several limitations. For example, usually long structures formation times are required and/or the presence of residual organic solvents is often found.

For these reasons, recently, supercritical fluids assisted processes have been implemented for biomedical applications; for example, supercritical foaming (Reinwalda et al., 2014), supercritical phase separation (Cardea et al., 2014; De Marco et al., 2014; Baldino et al., 2015a), supercritical gel drying (Daniel et al., 2012; Cardea et al., 2013; Baldino et al., 2015b). Another process strongly studied is the supercritical phase inversion. In this process, the supercritical fluid is used to induce the phase separation of the polymer solution. Compared with the traditional wet phase inversion method, advantages of this phase separation process can be:

(1) supercritical CO_2 (SC-CO_2) can dry the polymer membrane rapidly and totally without the collapse of the structure due to the absence of a liquid-vapour interface. The dry membrane can be obtained without additional post-treatment because there are no traces of organic solvents.

(2) It is easy to recover the solvent; the solvent dissolved in SC-CO_2 can be removed from gaseous CO_2 in a separator located downstream the membrane formation vessel.

(3) CO_2 is not toxic, not flammable and cheap.

Kho et al. (2001) used compressed CO_2 for the formation of Nylon 6 membranes. A 15% w/w Nylon 6 polymer solution was prepared by dissolving Nylon 6 in 2,2,2-trifluoroethanol (TFE). Thin film with thickness ranging from 150 to 250 µm were obtained. The process was performed at 35 °C with the final pressure up to 174 bar, for 30 min. These authors obtained uniform structures with cellular pores 0.4 µm in diameter. They described the structural gradient and pore characteristics of the membranes by a competition between a liquid-liquid (L-L) demixing, that leads to smooth, cellular pores, and solid-liquid (S-L) demixing, that leads to jagged or spiny

structures. The authors concluded that reducing the relative strengths of both the solvent and the non-solvent, led to membrane pore structures dominated by crystallization (S-L demixing), that is the thermodynamically favoured demixing process, rather than L-L demixing, which is kinetically favoured.

Another membranes formation process, in which SC-CO_2 is used as non solvent, has been presented by Matsuyama et al. (2002). The process consists of the introduction of CO_2 into a membrane formation cell through a buffer tank using a valve that connects the two vessels. After the system has been equilibrated (for 15 min) at the pressure of 130 bar, a second valve is opened and CO_2 flows in the cell to dry the phase-separated polymer solution. Matsuyama et al. (2001) studied the formation of thin polystyrene membranes analysing the effect of several process conditions (temperature, pressure, polymer concentration). They observed that the average pore size ranged from 8 to 35 μm changing pressure (from 75 to 150 bar), polymer concentration (from 15 to 30% w/w) and temperature (from 20 to 70 °C). In a successive work, Matsuyama et al. (2002) studied the influence of different solvents used during the formation of cellulose acetate membranes. The authors worked with all fixed process parameters: pressure, temperature and polymer concentration (130 bar, 35 °C, 15% w/w) and tested four solvents: acetone, methyl acetate, 1,3-dioxolane and 2-butanone. They noted that, as the mutual affinity between the solvent and CO_2 decreases, the membrane porosity and the pores size increases.

In this work, it has been evaluated the capability to generate HYAFF membranes by phase inversion assisted by supercritical fluids (i.e., supercritical carbon dioxide – SC-CO_2) and the effect of the process parameters on the generated structures has been studied.

2. Materials and methods

Materials

HYAFF, provided by the FAB (Fidia Advanced Biopolymers) S.p.A., is usually produced with different degrees of esterification (100, 75, 50%). In our tests, we used degrees of esterification of 100% and 75%. The esterification has essentially the purpose of decreasing the hydrophilicity of the polymer. The solvent used in the experiment was DMSO (Di-methyl-sulfoxide); it has been bought from Sigma Aldrich and the Carbon Dioxide from Morlando Group srl.

Membranes preparation

Polymer solutions were prepared by solubilizing HYAFF in DMSO; they were prepared at different HYAFF contents: 10, 12, 15 and 20% w/w. These values have been selected considering that: at lower HYAFF concentrations, too low viscosity solution were generated, whereas HYAFF concentrations higher than 20% w/w caused too high viscosity solutions.

The solution was distributed on stainless steel caps, with a diameter of 2 cm and a height of about 800 μm and, then, processed.

Membranes were produced in a home-made laboratory apparatus previously described (Baldino et al., 2014), at different process conditions. In Table 1 is reported the list of the experiments performed and the process conditions tested. The process conditions were selected considering previous works on similar materials (Baldino et al., 2014).

Table 1: List of the experiments performed at the different process conditions.

Test	Temperature [°C]	Pressure [bar]	% of Esterification	% w/w of HYAFF
1	35	100	100	10
2	45	100	100	10, 15
3	45	150	100	15
4	35	200	75	20
5	35	90	100	12
6	35	90	75	20
7	55	90	100	12

When operative temperature was reached, the caps were rapidly put inside the membrane preparation vessel (a 316 stainless steel vessel with an internal volume of 80 mL) to minimize the evaporation of the solvent. The vessel was closed and filled from the bottom with SC-CO_2 up to the desired pressure, with a high pressure pump (Milton Roy–Milroyal B, Pont-Saint-Pierre, France) for about 10 min. Then, the vessel was flush with CO_2 for 3 h, and, then, depressurized in about 30 min, collecting the dried membranes.

Scanning electron microscopy (SEM)

Membranes were cryo-fractured using liquid nitrogen (SOL, Milan, Italy); then, the samples were sputter coated with gold (AGAR Auto Sputter Coater mod. 108 A) at 30 mA for 150 s and were analyzed by a Scanning Electron Microscope (SEM) (mod. LEO 420, Assing, Italy), used to study membrane structure and pore size.

3. Results and discussion

To understand the mechanism of membranes formation during the phase separation process is necessary to refer to a ternary diagram Polymer-Solvent-NonSolvent (CO_2) (Fig. 1).

During the experimentation performed, HYAFF membranes have been obtained with different morphological characteristics, depending on the operating conditions. In particular, the first observation that it has been possible to put in evidence analyzing SEM images, is that the cellular structure, typical of membranes generated by supercritical phase inversion, has been never obtained during this work. This result is probably due to the size and shape of the miscibility gap in the HYAFF-DMSO-SC-CO_2 ternary diagram, that lead to different morphologies. Indeed, cellular structure is usually obtained when the demixing occurs in the upper part of the miscibility gap, between the binodal curve and the spinodal curve (path 1 in Fig. 1).

On the contrary, the morphologies obtained for HYAFF membranes, during this experimentation, have essentially been of two types:

- a) particulate-like, probably due to a binodal demixing in the zone below the critical point (path 3 in Fig. 1)
- b) bicontinuous, probably due to a demixing inside the spinodal curve (path 2 in Fig. 1); when a morphology due to a spinodal demixing is observed, it means that the extension of the spinodal area is quite large.
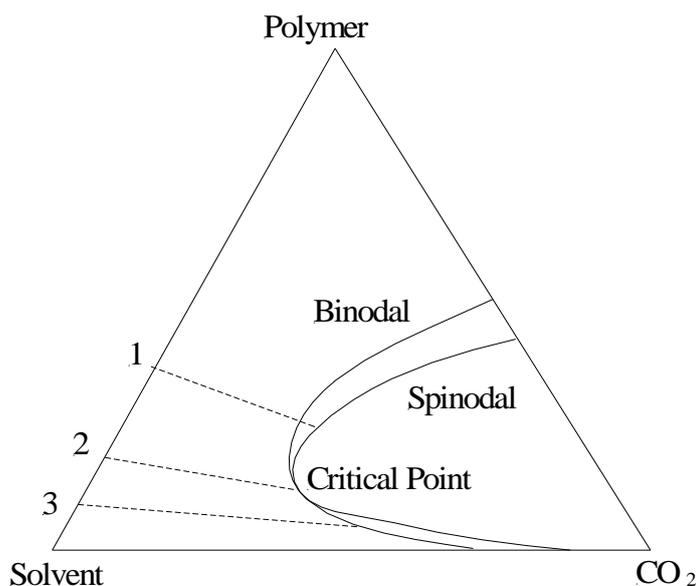
Figure 1: Polymer-Solvent-CO_2 qualitative ternary diagram.

We tried to give an interpretation of the results in analogy to what was performed in previous works about supercritical phase inversion process.

3.1 Effect of polymer concentration

Analyzing the effect of polymer concentration, we observed that an increase of the polymer concentration leads to a membranes morphology modification. Indeed, increasing the polymer concentration, the membranes passed from a particulate-like morphology (Fig. 2a) to a bicontinuous one (Fig 2b). It means that, considering a qualitative HYAFF-DMSO-SC-CO_2 ternary diagram, like the one proposed above (Fig. 1), the demixing point moved from a binodal demixing (path 3), occurring below the critical point, to a spinodal demixing (path 2), occurring in the spinodal zone. This result is quite obvious; indeed, starting from a more concentrated solution, the starting point of the mixture composition inside the ternary diagram is located in an

upper part of the Polymer-Solvent axis (from point 3 to point 2 in Fig. 1), favouring the spinodal demixing with respect to the binodal demixing.

a	b
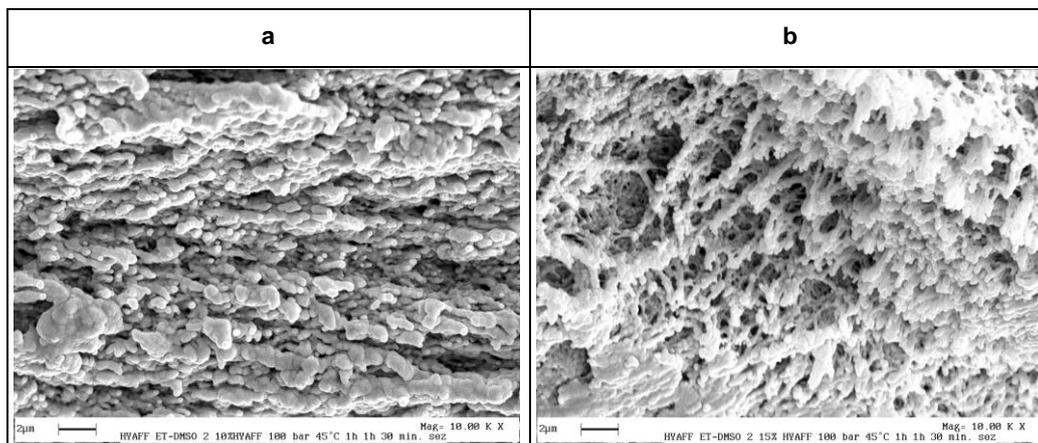
Figure 2: Effect of the polymer concentration on the membranes morphology (HYAFF 100% esterification) for the experiment at 100 bar and 45 °C; (a) 10% w/w, (b) 15% w/w HYAFF.

3.2 Effect of pressure

The effect of pressure on the HYAFF-DMSO-SC-CO$_2$ system has been also studied, from 90 to 200 bar. In particular, in Figure 3, HYAFF membranes obtained at 45 °C, 15% w/w and at 100 and 150 bar are reported.

a	b
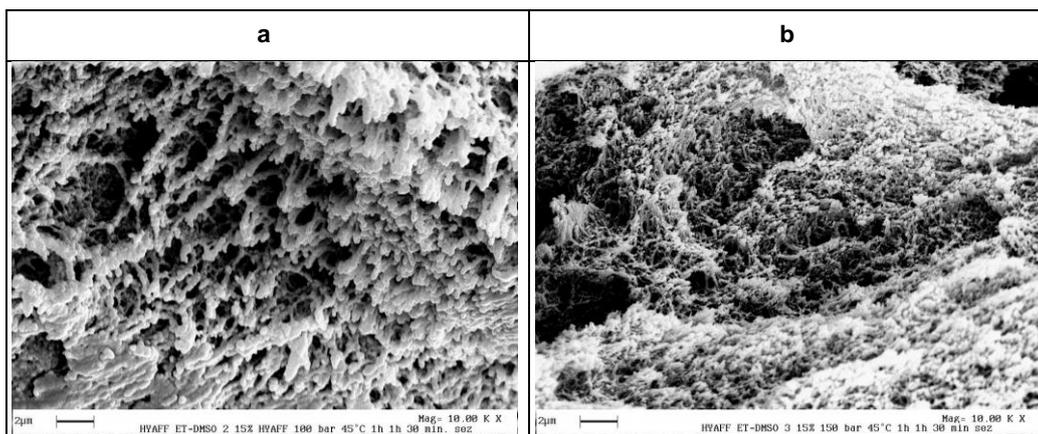
Figure 3: Effect of the operative pressure on the HYAFF membranes obtained at 45 °C and 15% w/w polymer totally esterified (100%); (a) 100 bar, (b) 150 bar.

As it is possible to observe from Figure 3, both membranes showed a bicontinuous morphology. However, the membrane obtained at the higher pressure (Fig. 3b) shows a denser mesh. This result can be explained by kinetic considerations based on the power solvent of SC-CO$_2$. Indeed, when the process occurs at a higher pressure, SC-CO$_2$ is characterized by an higher solvent power related to its density. Consequently, the demixing rate is higher and the drying of the membrane by SC-CO$_2$ is faster too. As a consequence, the pores created inside the membranes during the demixing process have less time to enlarge and a denser mesh is obtained.

3.3 Effect of temperature

Moreover, we tested the effect of temperature on HYAFF/ DMSO/ SC-CO$_2$ system; in Figure 4, the experiments performed at 35 °C and 55 °C are reported, both carried out at the pressure of 90 bar and at a concentration of 12% w/w.
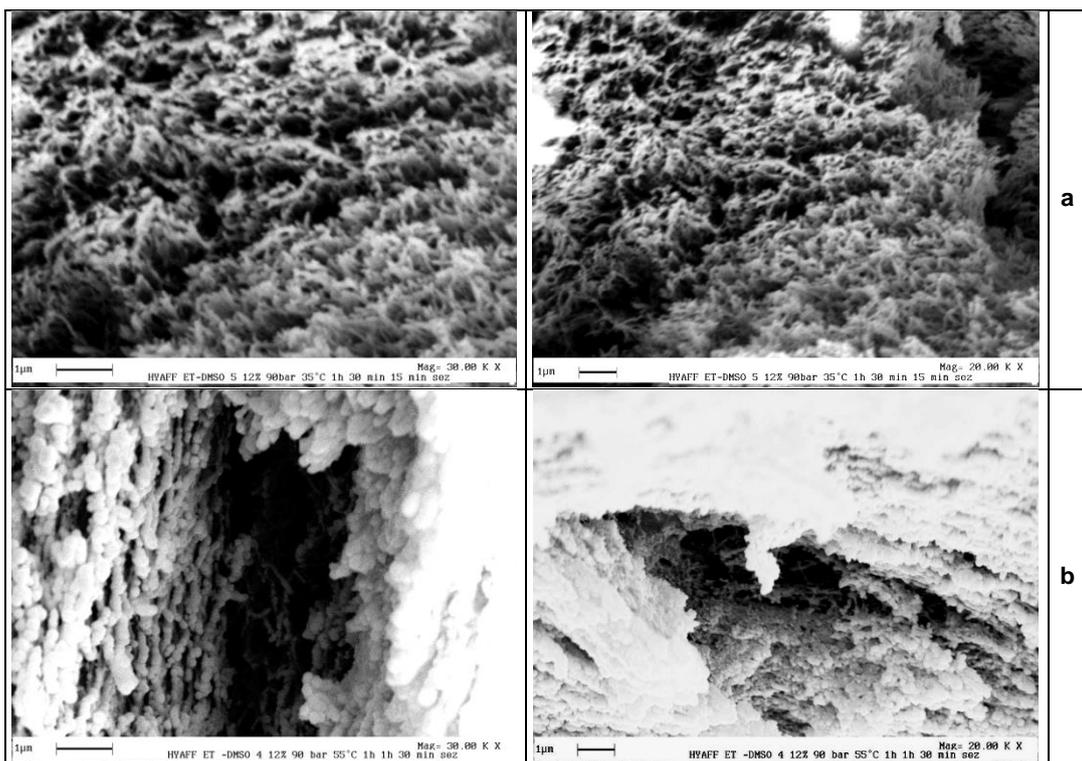
Figure 4: Effect of temperature on HYAFF membranes morphology, obtained at 90 bar and at 12% w/w polymer totally esterified (100%); (a) 35 °C, (b) 55 °C.

The membranes obtained at 35 °C show a bicontinuous morphology (Fig 4a); on the other hand, the membranes obtained at 55 °C appear to be characterized partially by a bicontinuous structure (but with a larger mesh) and partially by nanoparticles (Fig 4b). This result can be explained both by a reasoning similar to that carried out for the pressure effect (with regard to the enlargement of the bicontinuous mesh), both by considering thermodynamic aspects (regarding the formation of the particles).

For the explanation about the mesh of the structures, similar considerations to those reported for the effect of pressure can be used; indeed, an increase of pressure qualitatively has the same effect of a reduction of temperature.
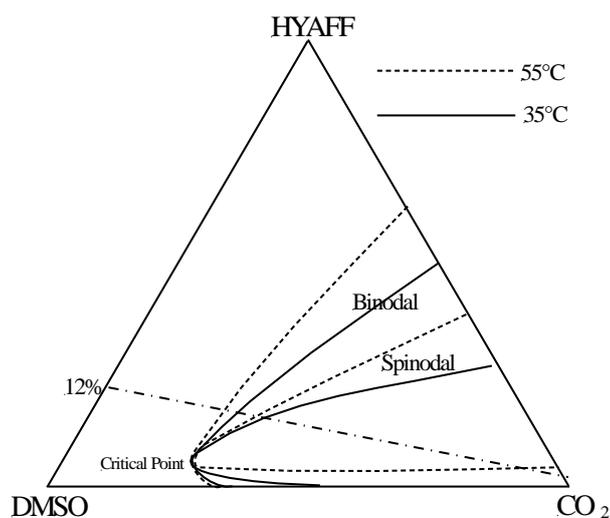
Figure 5: Modification of the miscibility gap in the ternary diagram caused by changing the process temperature.

Instead, from a thermodynamic point of view, it is possible to suppose that a decrease of temperature causes an extension of the miscibility gap in the ternary diagram (i.e., the solubility of HYAFF in DMSO-SC-CO_2 mixture decreases), as the one qualitatively reported in Figure 5. Consequently, starting from a fixed polymer concentration (i.e., 12% w/w in Fig. 5), the mixture composition path leads to a different demixing point varying the operative temperature. In particular, working at higher temperature, the system has more possibility to be demixed below the critical point, causing the polymer particles formation.

4. Conclusions

In this work, HYAFF membranes have been produced by phase inversion assisted by SC-CO_2. The effect of the process parameters on the generated structures have been investigated and we observed that operating at the higher pressure, smaller pores were obtained in the membrane; the same trend was observed when the operative temperature was reduced. Therefore, we demonstrated the flexibility of the supercritical process by changing the process parameters and the capability to design the membrane morphology: depending on the specific applications required, it is possible to modify the final morphology and pore size.

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