

Antimicrobial Membranes Produced by Supercritical Assisted Phase Inversion

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In this study, antimicrobial membranes of cellulose acetate (CA) loaded with potassium sorbate (Psb) were generated by supercritical assisted phase inversion for active packaging applications. To achieve appropriate membranes morphology, the starting solutions were prepared by dissolving Psb in water, and then, adding it to CA-Acetone solution and were processed at different pressures and temperatures.

The loaded membranes were analyzed by FESEM, EDX and UV/VIS spectrophotometer, to determine: membrane morphology, distribution of the active compound inside the polymeric matrix and Psb release rate, respectively.

The results indicate that, by changing the operative conditions (ranging from 150 to 250 bar, and 35 to 55 °C), cellular structures characterized by different pore size were obtained. EDX analyses showed as the active compound was uniformly distributed in the polymeric matrix at all process conditions tested. Moreover, Psb release time was influenced by membranes morphology, since the active compound diffusion was slowed down when the polymer matrix was denser. These results suggest that the antimicrobial membranes, prepared in this study, could be used as food packaging material achieving a controlled release of the active compound, improving the food quality and safety.

1. Introduction

Traditionally, antimicrobial agents are directly mixed into the initial food formulations. However, direct addition may result in a concentration decrease of the antimicrobial agent on the food surface, due to its diffusion into the interior parts of the food. Therefore, the minimum concentration required for the inhibition of the microbial growth may not be achieved and the antimicrobial compound cannot selectively target the food surface (Min and Krochta, 2005). In addition, the neutralization of the added agent, due to interactions with the food components, may occur (Appendini and Hotchkiss, 2006). For these reasons, the most important desired property of the antimicrobial packaging materials is the controlled release of the antimicrobial agent to the food surface (Uz and Altinkaya, 2011). Gemili et al. (2009) produced porous asymmetric cellulose acetate (CA) films for food packaging applications by traditional dry phase inversion, using lysozyme as antimicrobial component. They tried to control lysozyme release rate changing the degree of asymmetry and porosity of the films. Overall enzyme release times ranging between about 30 and 300 min were obtained. Uz and Altinkaya (2011) prepared CA mono and multilayer films including potassium sorbate (Psb) as antimicrobial agent by dry phase inversion technique. The results indicated that Psb release rate decreased as the CA content in the casting solution, the wet casting thickness and the drying temperature for both mono and multilayer films were increased.

Supercritical CO₂ (SC-CO₂) based processes have been frequently used to overcome the limitations of the traditional techniques; namely to control particle size (De Marco et al., 2013), to produce aerogels of different polymers, such as polystyrene (Daniel et al., 2012), chitosan (Cardea et al., 2013) and alginate (Baldino et al., 2014a), and organic solvent free nanofibres (De Marco and Reverchon, 2011) and scaffolds (Baldino et al., 2014b). Moreover, SC-CO₂ assisted phase inversion has been proposed to produce membranes of some polymers with controlled morphology and porosity, such as PMMA (Reverchon et al., 2006), PVA (Reverchon et al., 2007), PLLA (Reverchon et al., 2008) and Ethylcellulose (De Marco et al., 2014). Membranes loaded

with pharmaceutical (Cardea et al., 2010), catalyst (Cardea and Reverchon, 2011) and active agents for biomedical (Reverchon et al., 2009), tissue engineering (Reverchon and Cardea, 2012), drug controlled release from membranes (Cardea et al., 2014a), scaffolds (Cardea et al., 2014b) and food applications (Baldino et al., 2014c) have also been proposed using this technique.

More specifically, in a previous work CA membranes loaded with lysozyme have been prepared from CA-acetone solutions at various polymer concentrations (from 5 to 20 % w/w in CA), temperatures (between 35 and 55 °C) and pressures (from 150 to 250 bar) by SC-CO₂ assisted phase inversion method (Baldino et al., 2014c). The results were largely better than previous ones in the literature (Gemili et al., 2009). In particular, the presence of mobile and immobilized enzyme suggested that the membranes can be optimized for two different uses: if lysozyme migration was required to obtain a long range effect in the package (interaction with the head space), membranes that released a major quantity of mobile lysozyme were preferable. If a localized strong effect was required (direct interaction with the food), the membranes with the largest quantity of immobilized enzyme should be used obtaining cellular structure.

Starting from the encouraging results obtained for CA-lysozyme system, in the present work, the production of CA membranes loaded with Psb by SC-CO₂ assisted phase inversion is proposed, to obtain an active packaging device. The membranes will be analyzed in terms of porosity, pore size, Psb distribution and release.

2. Materials and Methods

Materials

Cellulose acetate, CA, (average Mn ca. 50 000 with Da acetyl content of 39.7%), acetone (purity 99.5%), Psb were bought from Sigma-Aldrich; CO₂ (purity 99%) was purchased from S.O.N. (Società Ossigeno Napoli, Italy); distilled water was produced in laboratory by ISCO mod. AUTOSTILL DST/5. All materials were processed as received.

Loaded membranes preparation

Polymer solutions were prepared by solubilizing CA in acetone; the solution containing Psb and water was separately prepared and, then, poured in the polymeric solution and mixed for 40 min. Solutions were prepared to produce CA contents of 15 % w/w, and a fixed quantity of 5 % w/w Psb and 5 % w/w water. 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., 2014c). 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-CO₂ 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 CO₂ for 3 h, and, then, depressurized in about 30 min, collecting the dried membranes.

Scanning electron microscopy (SEM)

Loaded membranes were cryofractured 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.

Membrane pore size analysis

Sigma Scan Pro 5.0 (Jandel Scientific, San Rafael, CANADA) and Origin 8.5 (Microcal, Northampton, USA) softwares were used to determine the average diameter of membrane pores. Images taken at various locations in the membrane were used for each calculation. We measured about 300 pores for each sample analyzed. Using Origin software, we first represented a histogram with the percentage of the pores having a given diameter; then, we performed a curve fitting to obtain the distribution curve.

Energy dispersive X-ray spectroscopy (EDX)

Antimicrobial membranes were cryofractured using liquid nitrogen and sputter coated with chrome (EMITECH K575X peltier cooled); then, they were analyzed by an Energy Dispersive X-ray spectroscopy (EDX) (INCA Energy 350, Oxford Instruments) to identify the elements that constitute the samples, representing Psb dispersion within the polymeric matrix.

Release test

Psb release kinetics were obtained measuring the increase of active compound amount in distilled water; the membrane was placed in a bottle containing 50 mL of distilled water at room temperature, stirred at 250 rpm. To determine the release rate from the membrane and the concentration of Psb, analysis was carried out in

continuous using a UV/VIS spectrophotometer Varian (mod. Cary 50), and reading the absorbance of the sample at 254 nm (that is the wavelength at which Psb shows maximum absorption).

3. Results and Discussion

In the first part of the work, we focused our attention on the effect of process parameters of SC-CO₂ assisted phase inversion on CA-Psb loaded membranes. In particular, ranges between 150 - 250 bar and 35 - 55 °C were explored. In Figure 1, SEM images related to CA-Psb membranes obtained at different process conditions (150 bar, 55 °C; 200 bar, 45 °C; 250 bar, 35 °C) are reported.

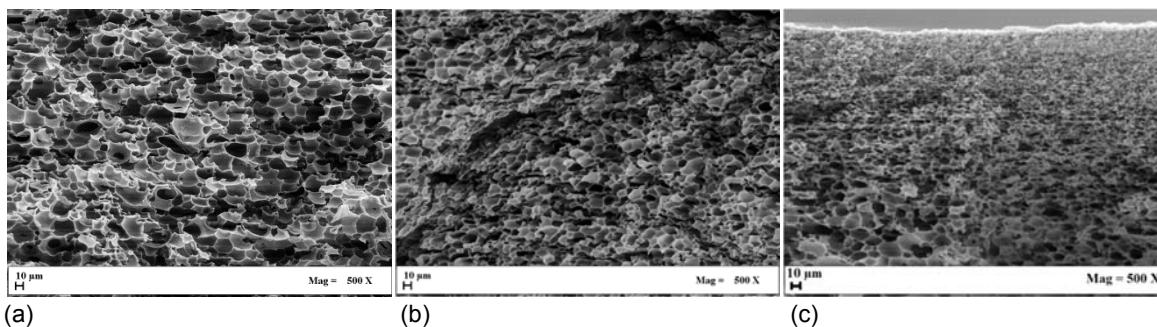


Figure 1: Effect of operative conditions on membranes morphology: a) 150 bar 55 °C, b) 200 bar 45 °C, c) 250 bar 35 °C

A cellular structure was obtained at all process conditions. In particular, increasing the operative pressure and decreasing the operative temperature (i.e., increasing the SC-CO₂ solvent power), the membrane mean pore size decreases. This result is well showed in Figure 2, where the pore size distribution of the various membranes are reported.

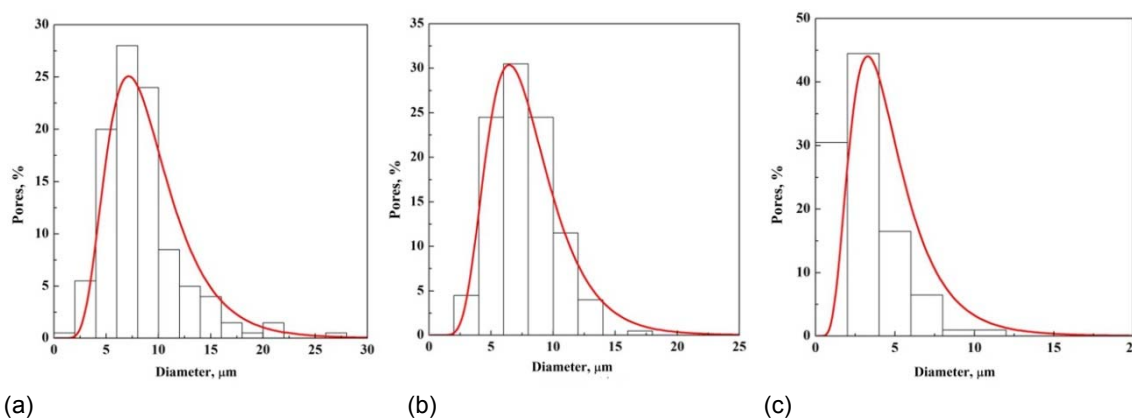


Figure 2: Effect of operative conditions on membranes pore size: a) 150 bar 55 °C, b) 200 bar 45 °C, c) 250 bar 35 °C

To explain the results obtained, we can refer to the theory of the traditional phase inversion process; indeed, as already reported in previous work (Reverchon et al., 2006), the SC-CO₂ phase inversion process theory can be related to the traditional ones. In this case, the formation of a cellular morphology indicates as the phase separation occurred in the upper part of the demixing gap (in the ternary system polymer/solvent/SC-CO₂), located between the binodal and the spinodal curves: nucleation and growth of droplets of the polymer lean phase with further solidification of the polymer rich phase was obtained.

To understand the effect of process parameters on mean pore size, we have to consider the kinetic of the SC-CO₂ assisted phase inversion process; indeed, the rate of phase separation depends on the SC-CO₂ solvent power. Increasing the pressure and decreasing the temperature (from a) to c) in Figures 1-2), the SC-CO₂ solvent power increases, making the phase separation faster. As a consequence, the polymer-lean phase has no time to grow during the membrane formation, causing the generation of smaller pores. In particular,

passing from 150 bar 55 °C, to 250 bar 35 °C, the SC-CO₂ density, that is directly related to SC-CO₂ solvent power, increases from 0.66 to 0.90 g/cm³.

Successively, to evaluate the dispersion of Pbs in the polymer matrix, EDX analysis was performed. In Figure 3, maps of Carbon (for CA - green) and Potassium (for Pbs - red) in a membrane obtained at 200 bar and 45 °C, are reported.

The maps of the elements generated by the EDX analysis show that the antimicrobial compound is uniformly dispersed within the polymeric matrix, since the areas in which Carbon is present (green) correspond to the areas in which Pbs (Potassium, in red) was also detected.

EDX results demonstrated the achievement of a necessary pre-requisite (homogeneous encapsulation of the bio-component) to obtain the controlled release of the active principle from the produced membranes.

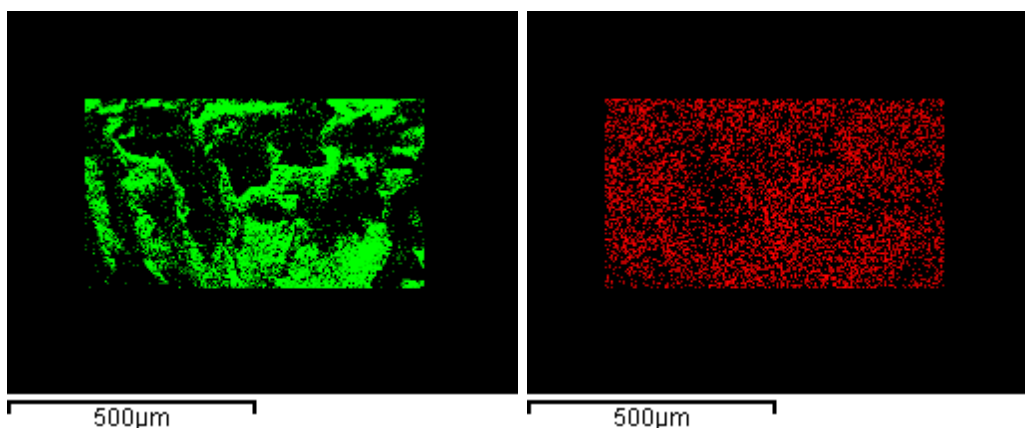


Figure 3: EDX analysis performed on membrane section: Potassium in red (characteristic element of Pbs), Carbon in green

In the last part of the work, we analyzed the kinetic of Pbs release from CA membranes, measuring the increase of Pbs concentration in distilled water, using the procedure described in Materials and Methods. As expected, a continuous increase of Pbs concentration was observed, as reported in the diagram in Figure 4, that is related to the release tests performed on Pbs loaded CA membranes produced at 15 % w/w CA and different pressures and temperatures. Pbs concentration (C_t) has been normalized to the maximum Pbs concentration (C_∞) to allow the comparison between the release curves.

Increasing SC-CO₂ solvent power, the diffusion rate of Pbs decreases, due to the increase of mass transfer resistance and a maximum of about 200 min is required to obtain the maximum concentration (at 250 bar and 35 °C).

This result can be explained looking at the membranes morphological study previously performed: 150 bar - 55 °C correspond to a SC-CO₂ density of 0.66 g/cm³, 200 bar - 45 °C and 250 bar - 35 °C correspond to a SC-CO₂ density of 0.81 and 0.90 g/cm³, respectively. As observed before, the increase of SC-CO₂ density produced CA membranes with smaller pore diameters and, therefore, the slowest diffusion rates were observed for the membranes produced at 250 bar and 35 °C.

Summarizing these results, when SC-CO₂ density increased, Pbs diffusion rate decreased; higher SC-CO₂ densities produced smaller pores and, therefore, also a decrease of the Pbs diffusion rates.

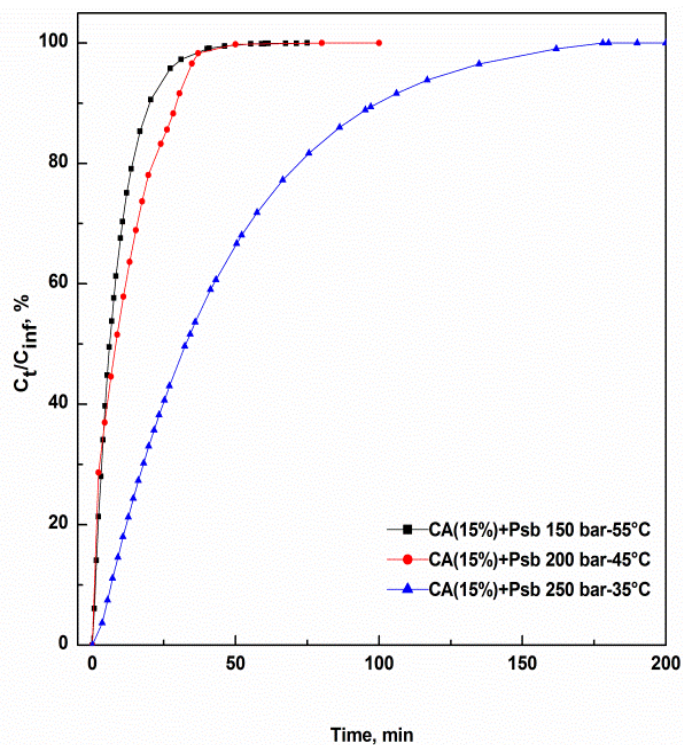


Figure 4: Effect of process parameters on PSB release from CA membranes

4. Conclusions and perspectives

SC-CO₂ assisted phase inversion has been used to generate CA membranes loaded with Psb for potential active packaging applications. We confirmed the promising results obtained with this process; in particular, we generated loaded membranes characterized by cellular morphology and homogeneous distribution of the loaded active principle. Moreover, the versatility of the process was evidenced: varying the operative parameters (pressure and temperature) it is possible to modulate the mean pore size and, as a consequence, the active principle release.

In future, it will be necessary to focus attention on the Psb release time, trying to increase it by the modification of other process parameters, such as polymer concentration and/or Psb concentration.

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