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Production of Cellulose Acetate Membranes Loaded with Quercetin by Supercritical CO_2 Phase Inversion

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Supercritical CO_2 phase inversion is one of the most promising techniques to generate polymeric membranes. In recent years, it has been also tested to generate loaded membranes to be used in several applications such as controlled release, catalysis, tissue engineering, water purification, etc. In this work, membranes of cellulose acetate were generated, loaded with a highly hydrophobic drug with antifungal properties, i.e., quercetin. Different process parameters were tested to verify their effect on the final morphology of the membranes and on the drug distribution along the membranes. In particular, polymer concentration in the starting polymeric solution was varied from 5 to 15% w/w, operative pressure ranged between 100 and 200 bar, operative temperature from 45 to 55 °C. Different membranes structures were obtained: finger-like and cellular-like; the effect of these different morphologies on quercetin release was also analyzed, finding as finger-like structure promoted a faster drug release (i.e., less than 200 min) with respect to cellular structures (i.e., up to 1400 min). Results confirmed the capability of supercritical CO_2 phase inversion process to generate loaded polymeric membranes and the high versatility of this process that allowed to generate different morphologies capable to control the drug release rate.

1. Introduction

Membranes are porous polymeric structures, the production of which is the subject of great interest. Depending on their characteristics, in fact, membranes can be used for different applications: in the field of food packaging, separation processes (reverse osmosis, nanofiltration, ultrafiltration, microfiltration), as thermal and electrical insulators, in the medical field and in catalysis. In particular, the production of membranes has great relevance in medical applications. Membranes can be used in controlled release of active ingredients, artificial organs, tissue regeneration, as a coating for medical devices, bioseparation, dialysis etc. [Stamantialis et al., 2008]. It has been estimated that the value of all the production of membranes intended for medical applications is far higher than that of all other applications [Baker et al., 2004]. One of the main reasons for the use of polymeric membranes in the pharmaceutical field is to ensure the controlled release of an active ingredient. Traditional forms of delivery of active ingredients, such as tablets or injections of solutions, are characterized by peaks, often above the required dose [Reverchon et al. 2009]. The use of membranes intended for the controlled release of the active ingredients has several advantages: elimination of repeated dose administration leading to an improvement in patient compliance, better regulation and control of the release rate of the active ingredient, reduction of the variability of the ingredient concentrations active in the blood; moreover, it is possible to reduce the degradation of the active ingredient molecules and the loss of their therapeutic activity before the active ingredient is released. Reducing the number of doses administered is also important to reduce as much as possible the phenomenon of "first pass metabolism", a phenomenon of the metabolism of some drugs which occurs when the bio-availability of the drug is significantly reduced before it reaches and is distributed throughout the body. The release of the active ingredient is controlled by various mass transport mechanisms, such as diffusion, erosion, “swelling” or osmosis, which depend on the membrane formation process, on the material properties (composition, porosity), and on the properties of the active ingredient, such as solubility and molecular

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weight [Reverchon et al., 2006]. To create a system intended for the controlled release of an active ingredient, a meticulous study is needed to carefully choose a polymer/active ingredient pair that satisfies the searched criteria. Many synthetic and natural polymers have been investigated for use in controlled release systems and some have been approved for their commercial use by the Food and Drug Administration (FDA). The most used natural polymers are polysaccharides with high molecular weight such as alginates, chitosan, agarose, carrageenan, or proteins such as collagen, albumin, gelatin, elastin [Baldino et al., 2015]. However, natural polymers have more limitations in mechanical properties and processability than synthetic polymers. For this reason, more attention has been paid to the use of synthetic polymers for controlled release systems. Among the polymers used are polyesters, polyamides, polyurethanes, acrylic polymers, and polymers derived from cellulose, such as cellulose acetate. Polymeric membranes can be made using traditional techniques, including:

• NIPS (Non Solvent Induced Phase Separation): the solution, formed by a polymer and a solvent is immersed in a "non-solvent" for the polymer, in which the contact between the solvent and the non-solvent causes the phase separation of the solution and the precipitation of the polymer, generating porous structures.

• TIPS (Thermally Induced Phase Separation): solid-liquid phase separation occurs when the temperature is low enough for the solution to freeze. After the frozen solvent is removed, the remaining spaces become pores.

• Solvent-evaporation: in this case, the parameters that must be considered are the temperature, relative humidity and the drying time.

However, the traditional techniques used have numerous limitations that negatively influence their use: long process times; residues of organic solvents, which must be removed through very expensive post-treatment techniques, as any residues can compromise the use of membranes in the biomedical, pharmaceutical and food packaging fields; production of asymmetric structures, with closed and irregular pores; they are not very versatile processes: it is difficult to change the morphology of the membrane and to modulate the size of the pores by changing the process parameters.

Due to the limitations due to traditional techniques, innovative methods have been proposed for the production of porous structures; one of these involves the use of supercritical CO_2 (SC-CO_2), and is called phase inversion assisted by supercritical CO_2. In this technique, a polymer solution comes in contact with the SC-CO_2 which captures the organic solvent, causing the phase separation phenomenon and producing a porous membrane [Cardea et al., 2014, Cardea et al., 2006, Baldino et al. 2019], similar to conventional phase inversion. The use of SC-CO_2 has many advantages: it is inexpensive, non-toxic, non-flammable, and can be reused several times before being released into the atmosphere, with a reduced environmental impact compared to traditional techniques. In addition, the use of SC-CO_2 manages to eliminate one of the main limitations posed by traditional techniques: the membranes obtained do not have to be subjected to post-treatments, which in traditional techniques turned out to be very expensive, as the solvent used to dissolve the polymer is completely extracted from the SC-CO_2 and can be easily removed following the gasification of the CO_2 after depressurizing it. The use of SC-CO_2 allows to improve process performance: SC-CO_2 can form polymeric membranes rapidly, without causing their collapse because there is no liquid-gas interface thanks to the formation of a supercritical mixture with the solvent it is extracting. Phase inversion using SC-CO_2 allows to generate symmetrical membranes and to modulate the morphology of the membranes simply by varying the process parameters. The fundamental parameters in the formation of membranes by supercritical phase inversion are the concentration of the polymer, the process pressure and temperature and the type of solvent used.

The polymeric membranes produced can be loaded with a certain active ingredient: in particular, great attention has been paid to flavonoids. Flavonoids have in common two benzene rings connected through a heterocyclic pyrone ring. Animals are unable to synthesize flavonoids, which for this very reason can only be found in plants. Quercetin (figure1) is obtained as a condensation product of p-glycosides and is found in large concentrations in fruits and vegetables, such as apples, cherries, broccoli, grapes, lettuce, onions, tomatoes, blueberries and leeks, resulting be one of the major flavonoids in the human diet.

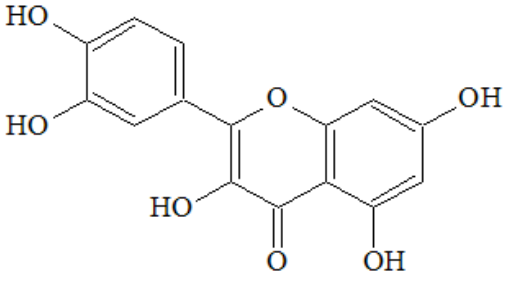

Figure 1: Chemical structure of quercetin

Quercetin has beneficial effects on health thanks to its antioxidant property, i.e. thanks to its ability to capture free radicals and transition metal ions. In vitro experiments have shown that quercetin can be effective in treating different types of cancer and can be combined with other types of anticancer drugs to reduce their dose and, consequently, their side effects. Studies have been conducted on its anti-inflammatory, antiangiogenic and antiproliferative activity, its hepatoprotective effect. Quercetin has been studied for the treatment of ovarian cancer, breast cancer, pancreatic cancer, cervical cancer, colon cancer. Its antimicrobial activity has also been studied [Gurana et al., 2019].
For these reasons, the purpose of this work is to verify the capability to produce membranes of cellulose acetate loaded with quercetin, using the phase inversion assisted by supercritical CO_2. Moreover, the effect of process parameters has been studied.

2. Results

Cellulose acetate membranes were prepared starting from the solubilization of polymer concentration ranging between 5 and 15% in acetone. Quercetin was added at different concentrations by weight (5% and 10%) with respect to cellulose acetate. The experiments were performed at different operative conditions: 200 bar / 45°C and 100 bar / 50°C.
SC-CO_2 assisted phase inversion was performed using a laboratory plant consisting of a 316 stainless-steel cylindrical high-pressure vessel with an internal volume of 200 mL, equipped with a high-pressure pump (mod. LDB1, Lewa, Leonberg, Germany) used to deliver liquid CO_2. Pressure in the vessel was measured using a test gauge (mod. MP1, OMET, Lecco, Italy) and regulated using a micrometering valve (mod. 1335G4Y, Hoke, Spartanburg, USA). Temperature was regulated using PID controllers (mod. 305, Watlow, Corsico, MI, Italy). At the exit of the vessel, a rotameter (mod. D6, ASA, Sesto San Giovanni, MI, Italy) was used to measure CO_2 flow rate.

a. Effect of polymer concentration

In the first part of the work, the effect of polymer concentration on the final structure was studied. Changing the polymer concentration from 5 to 15%, different membranes morphologies were obtained:

- at 5% w/w, particulate membranes were obtained (figure 2a);
- at 10% w/w, finger-like structures were obtained (figure 2b);
- whereas, at 15% w/w, cellular structures were generated (figure 2c).

These results confirmed the high versatility of the process that allowed to produce very different membranes.

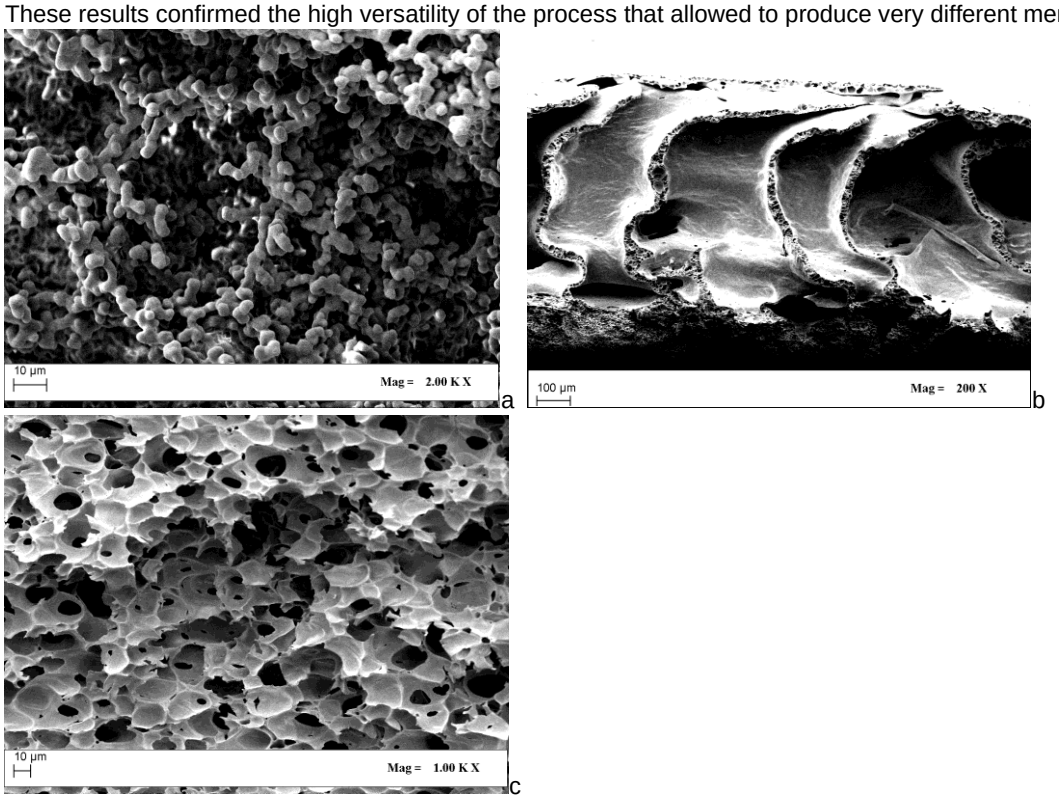

Figure 2: Cellulose Acetate-Quercitin loaded membranes obtained at different polymer concentrations: a) 5% w/w, b) 10% /w, c) 15% w/w.

The presence of quercetin did not affect the final structures. Indeed, performing experiments without quercetin in the starting solutions, we verified as the same membranes morphologies were obtained. Probably, quercetin did not influence the mechanisms involved during the phase inversion that led to the structures reported above.

b. Effect of Pressure and Temperature

Subsequently, our attention was focused on the effect of pressure and temperature. Various experiments were conducted at two different operating conditions, 200 bar and 45°C, and 100 bar and 50°C. The effect of the operating conditions on the final morphology of the membranes was studied. Indeed, as the operating conditions varied, the density of the SC-CO_2 varied significantly:

• 200 bar, 45°C: density = 0.81 g/cm^3,

• 100 bar, 50°C: density = 0.39 g/cm^3.

The solvent power of the SC-CO_2, which influences the kinetics of the process, is mainly linked to its density, which increases with increasing pressure and decreasing temperature. When the density of the SC-CO_2 decreases, and therefore its solvent power decreases, the phase inversion becomes slower and the polymer-lean phase has more time to grow, thus generating larger pores. The effect of the operating conditions can also be observed in the membranes produced starting from 10% w/w: although they all have a finger-like structure, as the pressure increases and the temperature decreases (i.e. as the density of the SC-CO_2) the size of the fingers decreased.

For the membranes at 15% w/w, an analysis of the mean size of the pores and their distribution was made, and the results obtained are reported in table 1:

Table 1: 15% w/w Cellulose Acetate membranes characteristics obtained at different operative conditions (100 bar/50°C and 200 bar/45°C)

Operative conditions	Quercetin [% w/w]	Mean pore size [µm]	Standard deviation [µm]
100 bar 50°C	5%	9.33	3.09
200 bar 45°C	5%	5.02	2.32
100 bar 50°C	10%	11.12	3.34
200 bar 45°C	10%	6.05	2.52

As the density of the SC-CO_2 increased, the mean pore size decreased (from 9.33 µm to 5.02 µm for membranes loaded with 5% w/w quercetin, and from 11.1 µm to 6.05 µm for membranes loaded with 10% w/w of quercetin), confirming that the process kinetics is influenced by the operating conditions. Furthermore, the distribution of the pores narrowed as the density of the SC-CO_2 increased: observing the membrane produced starting from 15% w/w of cellulose acetate with 10% w/w of quercetin at 200 bar and 45°C at a smaller magnification, the structure of the membrane is uniform and regular (Figure 3).

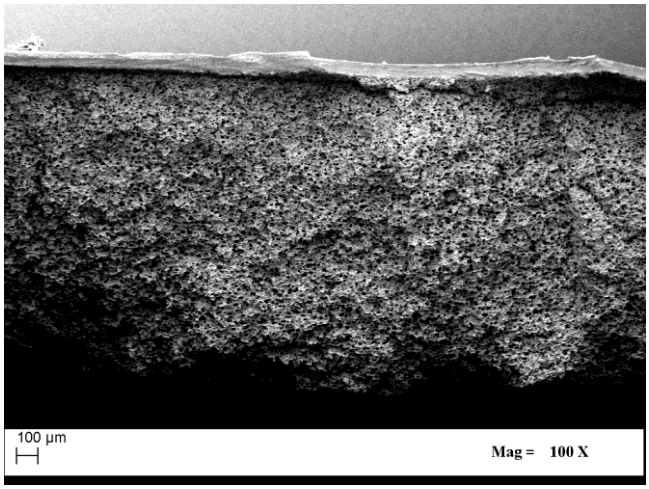

Figure 3: SEM image of 15% w/w cellulose acetate membrane with 10% w/w of quercetin, obtained at 200 bar and 45°C

c. Release kinetics of quercetin

In the last part of this work, the release kinetics of quercetin from Cellulose Acetate membranes was studied. The maximum concentration of quercetin was normalized to be able to compare the obtained curves.
The instrument used for the release tests performed on the Cellulose Acetate - Quercetin membranes was the Varian Cary® 50 UV/VIS spectrophotometer. The quercetin release tests were performed by measuring the concentration of the active ingredient in PBS (phosphate buffer, @ pH 7.4), at room temperature. The loaded membrane was placed in 25 mL of PBS, to simulate the human environment, and a wavelength of 375 nm was used to read the absorbance of the sample (this is the wavelength at which quercetin shows the maximum absorbance). First, a calibration line was determined, which relates the absorbance measured by the instrument and the concentration of the active ingredient in the solution. Using solutions of the active principle as it is dissolved in PBS at different known concentrations, the calibration line of quercetin in PBS was obtained from the experimental data, the equation of which is shown below Eq(1):

$$A=0.00553 * C \qquad Eq(1)$$
$$R^2=0.876$$

In all the experiments carried out, the membranes produced starting from 5% w/w of Cellulose Acetate in the starting solution have a particle structure; consequently, they do not have the characteristic porous matrix required for controlled release applications, and for this reason they were not analysed. In the other cases, the release curves show an asymptotic exponential trend; it can be deduced that the release mechanism is the same for membranes of Cellulose Acetate at 10 and 15% w/w, i.e., diffusive type. But, passing from a finger morphology to a cellular morphology, the release of quercetin was found to be sensibly slower (figure 4): this is due to the increase in resistance to the mass transport offered by the polymeric matrix in the case of membranes with higher polymer concentration (i.e., cellular structure). Moreover, increasing the SC-CO_2 density, it is possible to furtherly control the release rate: indeed, at higher SC-CO_2 density, smaller pores were obtained that caused a slower release (i.e., increase of resistance to the mass transport)

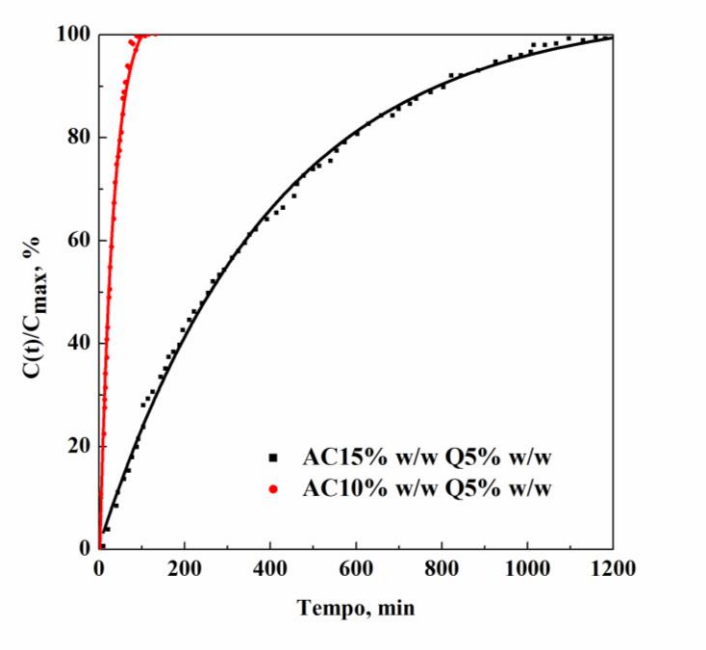

Figure 4: Quercetin release data from membranes produced at 200 bar 45°C, at 10% and 15% w/w of polymer, and with 10% w/w of quercetin loading.

3. Conclusions

In this work, cellulose acetate membranes loaded with quercetin were produced and characterized by SC-CO_2 assisted phase inversion. It was possible to modulate the morphology of the membranes and the average pore size as the studied process parameters varied. The versatility of the process was also confirmed by the release kinetics, one of the main requirements for controlled release systems of active ingredients: it is possible to obtain

longer or shorter releases by modulating the mean pore size of the membrane and its morphology. As the polymer concentration and the density of the SC-CO_2 increase, the membranes produced present a cellular structure and a smaller average pore size: this influences the release kinetics which is slower. In fact, the release of quercetin occurs in longer times with the membranes produced starting from 15% w/w Cellulose Acetate, obtained at 200 bar and 45°C (up to 1200 minutes).

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