EFFECTS OF SUPERCRITICAL CARBON DIOXIDE PROCESSING ON THE PROPERTIES OF CHITOSAN-ALGINATE MEMBRANES

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Abstract

Chitosan-alginate membranes with different porosities designed to be used as wound dressings or scaffolds were processed in supercritical CO₂ (scCO₂) with the aim of improving their physicochemical properties. The membranes were characterized before and after processing at 100 or 300 bar and 45°C for 2 hours with a depressurization rate of 5 bar/min. The results show that after processing, thickness, real density and porosity increased up to 68%, 100% and 167%, respectively, and surface area decreased up to 47%. Because of changes in structure, water vapor sorption and permeability increased up to 53% and 84%, respectively. Stabilization of the chitosan-alginate complex was noticed after processing with scCO₂ according to thermal analysis. The results indicate that supercritical CO₂ processing made the membranes more adequate for use as wound dressings.

Keywords: Chitosan-alginate membranes, biopolymers, supercritical CO₂ processing, biomedical applications

1. Introduction

Chitosan and alginate are among the polysaccharides most frequently studied for biomedical applications [1]. Chitosan is a linear polysaccharide, composed of units of

glucosamine and N-acetyl glucosamine. It obtained by deacetylation of chitin, a polysaccharide found in the exoskeleton of crustaceans, insects and also in fungi. Chitosan is recognized by its unique properties, such as non-toxicity, biocompatibility and biodegradability [2]. Alginate is a linear polysaccharide consisting of alternating blocks of (1,4) β -D-mannuronate (M) and α -L-guluronate (G). For commercial purposes, it is extracted from brown seaweeds [2]. Alginate can absorb water/body fluids up to 20 times its weight providing a moist wound-healing environment [3].

The formation of chitosan-alginate polyelectrolyte complex (PEC) is a result of strong electrostatic interactions between amino groups of chitosan and carboxyl groups of alginate. Compared with alginate or chitosan gels alone, the PEC possesses many improved physicochemical properties such as higher stability to pH changes and improved effectiveness as controlled-release systems [1,2]. This PEC can be used in many advanced biomedical applications, such as wound dressings, drug delivery and tissue engineering [2].

In previous works [4,5], the development and characterization of porous chitosan-alginate PEC membranes obtained through the addition of a non-ionic surfactant Pluronic F68 compatible with the human skin [6] was described. Pore formation upon the addition of Pluronic F68 to the PEC occurred due to its high hydrophilicity and capacity of forming foams in aqueous solutions under stirring. Bueno *et al.* [5] found that the higher the amount of surfactant added, the higher the air retention in the polymeric mixture and, consequently, the higher the pore size of the dried biomaterial. These membranes, produced through a simple and low-cost method, were considered as potential candidates for wound dressings and for the use in tissue engineering as skin scaffolds.

Supercritical CO₂ (scCO₂) technology may be employed as a polymer plasticizing/swelling strategy, with the capability of changing the porosity and consequently the permeability of the material to gases and moisture. Polymer matrices incorporating bioactive agents may be additionally modified regarding the kinetic behavior release of the compound. Changing process conditions such as CO₂ pressure, residence time and depressurization rate may then be used to attain desired properties of porosity, permeability and release kinetics [7-9].

Only a few works about CO₂ processing of biopolymers are available in the literature, what can be probably attributed to the heterogeneity of the natural matrices which limits the study of processing effects [7-9]. In addition, natural polymers such as cellulose, chitin, gelatin, and chitosan present some crystallinity and show little or no absorption of CO₂ in the solid state. However, and since chitosan-alginate polyelectrolyte complexes (PEC) are more stable, processing effects may be more effective in this type of matrix. Considering the benefits about using scCO₂ technology to process polymer matrices, and the lack of data regarding the use of this strategy, CO₂ processing of chitosan-alginate PEC membranes could be a novel approach to modify, control, and even improve the biomaterial properties.

Therefore, the main purpose of this work was to determine the effects of this process on the physicochemical properties of membranes having different porosities.

2. Materials and Methods

2.1 Materials - Chitosan-alginate membranes were prepared using chitosan from shrimp shells, having a deacetylation degree of 95 ± 1 % as determined by a titrimetric method, and low viscosity sodium alginate, both from Sigma-Aldrich (United States). The molar masses of the polymers were determined by viscosimetry (capillary viscosimeter Ostwald-

Cannon-Fenske, n.200), being equal to 1.26×10^6 g/mol for chitosan and 4.69×10^4 g/mol for alginate.

Pluronic F68, currently available as Poloxamer 188, was obtained from Sigma-Aldrich (United States), calcium chloride dihydrate and sodium hydroxide were obtained from Merck (Germany), acetic acid was obtained from Synth (Brazil) and CO₂ at 99.998% was obtained from Praxair (Spain).

2.2 Preparation of the membranes

Chitosan-alginate (1:1) porous membranes were prepared in the presence of Pluronic F68 at 2% and 10% (w/w) in relation to the total polysaccharides mass. For the preparation of the membranes, 90 mL of 1% (w/v) chitosan in 2% (v/v) aqueous acetic acid were added to 180 mL of 0.5% (w/v) alginate containing or not Pluronic F68 at 2% or 10% (w/v) at 200 mL/h, with the aid of a peristaltic pump (model Minipuls 3, Gilson). The reaction occurred in a jacketed stainless steel tank with internal diameter of 10 cm and height of 20 cm at a stirring rate equal to 500 rpm (mechanical stirrer model Q-251D, Quimis, with a 4 cm in diameter three tilted-blade propeller), at 25°C (temperature maintained with a thermostatic bath Q-214M2, Quimis). The final suspension was homogenized at 1000 rpm for 10 minutes. Afterwards, 1M NaOH was added to increase the pH to 5.3, under the same stirring rate, for 10 minutes. Then, 3.6 mL of 2% (w/v) CaCl₂ aqueous solution were added to crosslink the alginate carboxyl groups that were not bounded to the chitosan amino groups and the suspension was stirred for 10 minutes more. The amount of CaCl₂ added was previously established by Rodrigues [10] aiming to ensure carboxyl groups crosslinking while avoiding excess gelification and consequent PEC precipitation. The membranes were prepared by casting: the polymeric mixture was transferred in equal mass proportions to

two polystyrene Petri dishes having diameters of 15 cm, then the material was dried in an oven with air circulation (model 410D, Nova Ética) at 60°C until achieving constant weight. The membranes were removed from the Petri dishes and immersed for 30 minutes in 150 mL of 2% (w/v) CaCl_2 for further crosslinking. The second crosslinking step is crucial because, if the membranes are directly exposed to water after the oven-drying step, the free alginate chains dissolve, resulting in a heterogeneous membrane [10]. The membranes were then washed twice for 30 minutes in 200 mL of deionized water to remove the residual acetic acid not eliminated during the casting process, deemed toxic to the selected applications, and dried at 37°C for 6 hours, with their borders attached to the Petri dishes to avoid shrinking. Samples were designed as P0%, P2% and P10% according to their Pluronic F68 content.

2.3 Supercritical CO_2 processing

The employed supercritical apparatus was already described in literature [7]. The system was comprised of a high pressure liquid CO_2 pump, a stainless steel high pressure cell with approximately 10 cm³ of internal volume, a temperature-controlled water bath (Haake DC30, Thermo Electron Corporation) and a pressure transducer (Datum 2000TM, Setra).

The cell, containing 1 cm x 1 cm membrane samples in stainless steel supports (as shown in Figure 1), was pressurized at 100 or 300 bar at 45°C for 2 hours. At the end of the process, depressurization of the system was carried at 5 bar/min. Different pressures were employed since this operational condition strongly influences both scCO_2 density and solubilizing capacity, which could assist on the definition of future conditions for the impregnation of bioactive compounds in the membranes. Before being exposed to CO_2 , the samples (P0%, P2% and P10%) were kept at 20% relative humidity for

48 hours before processing.

2.4 Physical and chemical characterization

Since the obtained membranes are hygroscopic and may dry in excess at very low relative humidity, turning too brittle, the samples were kept at 20% RH for 48 hours before most of the characterization tests, namely, thickness, surface area, real density, porosity, water vapor sorption, water contact angle and simultaneous data thermal analysis (SDT). This condition was deemed as adequate for storing the samples for commercialization purposes. Before the scanning electron microscopy (SEM) analysis, it was necessary to keep the samples in a desiccator with silica gel for 48 hours, since water may interfere negatively in the results. Finally, before water vapor permeability tests, the samples were previously kept at 50% RH for 48 hours according to Rhim [11] and Moraes and Beppu [12], aiming at simulating the conditions of use on the lesioned skin as dressings.

2.4.1. Aspect

The aspect of the membranes was inspected visually regarding color change and material integrity.

2.4.2. Morphology

Detailed analysis of the membranes morphology was performed by scanning electron microscopy (SEM) with a microscope (model LEO 440, Leica) at 10kV and 50pA. The samples were previously coated with an ultra-thin layer of gold (92Å) in a mini Sputter coater (SC 7620, VG Microtech).

2.4.3. Thickness

The thickness of samples was measured with a digital micrometer(model MDC-25S, Mitutoyo), being reported as the average of 10 individual measurements.

2.4.4. Surface area, real density and porosity

The surface area of the membranes was measured by N₂ adsorption(Micrometrics ASAP 2000 V2.04.), and calculated with the BET equation (Equation 1):

$$\frac{1}{V_a \left(\frac{P_0}{P} - 1 \right)} = \frac{C-1}{V_m C} \times \frac{P}{P_0} + \frac{1}{V_m C} \quad (\text{Equation 1})$$

where P (Pa) is the partial vapor pressure of N₂ gas in equilibrium with the surface at 77.4 K(boiling point of liquid N₂), P₀ (Pa) is the saturated pressure of N₂, V_a (mL) is the volume of gas adsorbed at standard temperature and pressure (273.15 K and 1.013x10⁵ Pa), V_m (mL) is the volume of gas adsorbed at standard temperature and pressure to produce an apparent monolayer on the sample surface, C is a dimensionless constant that is related to the enthalpy of adsorption of the adsorbate gas on the powder sample.

After plotting $1/[V_a(P_0/P - 1)]$ against P/P_0 , V_m is calculated by linear regression and the specific surface area is calculated according to Equation 2:

$$S = \frac{V_m N a}{m \times 22400} \quad (\text{Equation 2})$$

where N (mol⁻¹) is the Avogadro constant, a (m²) is the effective cross-sectional area of one N₂ molecule and m (g) is the mass of the sample.

The real density was measured by helium pycnometry(Accupyc 1330Micrometrics, USA).

The total porosity was calculated according toEquation3:

$$P_t = 1 - \rho_{\text{apparent}} / \rho_{\text{solid}} \quad (\text{Equation 3})$$

where ρ_{apparent} (g/cm^3) is the apparent density (calculated as the ratio between the mass and the volume of each sample), and ρ_{solid} (g/cm^3) is the real density measured by helium pycnometry.

2.4.5. Water vapor sorption

Water vapor sorption was evaluated gravimetrically, according to Dias *et al.* [8], by measuring the gradual increase in weight of 1 cm x 1 cm samples (in 95% RH at 32°C). The experiments were performed in triplicate. Vapor sorption capacity was calculated according to Equation 4, in which W_t is the weight of the sample at time t and W_0 is the initial weight of the sample.

$$V_s = \frac{(W_t - W_0)}{W_0} \times 100 \quad (\text{Equation 4})$$

2.4.6. Water vapor permeability

Water vapor permeability was evaluated by a gravimetric method adapted from ASTM E96-95 [13]. The experiments were performed in triplicate with 1.2 cm x 1.2 cm samples, which were fixed at the top of small flasks containing silica gel, resulting in an available area for contact with air equal to 0.6362 cm^2 . The flasks were stored at 50% RH at 32°C and had their weights measured periodically during 24 hours. Water vapor permeability (V_p) was calculated according to Equations 5 and 6:

$$V_p = \frac{T_v \cdot l}{P_{\text{vap}} \cdot \Delta RH} \quad (\text{Equation 5})$$

$$T_v = \frac{W_v}{t \cdot A} \quad (\text{Equation 6})$$

where W_v is the weight of the vapor that permeated through the sample at time t , A is the area of the sample exposed to the atmosphere, l is the thickness of the sample, P_{vap} is the

vapor pressure and ΔRH is the difference between the relative humidity above and below the sample.

2.4.7. Water contact angle

Measurements of static contact angle of water were performed with equipment OCA20 (Dataphysics) after compacting the samples with a manual press for about 1 min, to avoid the influence of porosity and surface irregularities. The results were the average of twenty measurements for each sample.

2.4.8. Simultaneous data thermal analysis (SDT)

The thermal and calorimetric behavior was determined on a Simultaneous data thermal analysis (SDT) equipment (Q100, TA Instruments, New Castle DE, USA) using standard alumina crucibles, heating at 10 °C/min from 25°C up to 700 °C). The instrument was calibrated with indium. The results are expressed as the average of at least two independent samples.

3. Results and Discussion

3.1. Aspect, morphology and thickness

The membranes did not show evidence of major changes in color or in integrity after being exposed to the scCO₂ in the different conditions when evaluated with the naked eye. The typical morphologies of the membranes analyzed by SEM are shown in Figure 2. It is possible to notice that the obtained membranes have a porous and lamellar structure, and also that the pores do not cross the entire structure of the matrix. This characteristic favors their application as dressings for the treatment of skin lesions, since

the penetration of microorganisms into the wound would be hindered[4, 14].

The thickness of a membrane also has great influence on its performance as a biomaterial. The measured thickness values ranged from 0.12 to 0.84 mm depending on the Pluronic F68 proportion and on the pressure conditions (Table 1). For the treatment of skin lesions, it is recommended that the thickness of the dermis temporary substitute is thinner than the human dermis, which varies from 0.5 to 2 mm, depending on age, sex and body region [15]. Therefore, regarding thickness, the membranes obtained in this work can be considered adequate for the treatment of skin injuries before or after scCO₂ processing.

Samples prepared in the presence of growing amounts of Pluronic F68 were significantly thicker than those obtained with smaller amounts of surfactant, what can be explained by the greater incorporation of air into the polymeric mixture promoted by the surfactant under mechanical stirring, as also noticed by Bueno *et al.* [5]. Therefore, the membranes prepared in the presence of higher Pluronic F68 proportions have bigger pores and greater voids between the transversal layers of the membranes, as can be observed in Figure 2.

There were no significant changes in thickness after scCO₂ processing for sample P0%, while the average thickness increased up to 75% for sample P2% and 68% for sample P10% (Table 1). However, the increase in the average thickness with the increase of pressure observed could not be confirmed by SEM (Figure 2), probably because the analysis was performed with small samples (1 cm x 1 cm), which only represent a section of the membrane.

The increase in thickness of samples P2% and P10% may be related to the presence of Pluronic F68, which, according to Bhomia *et al.* [16], melts in the processing conditions employed. The melting of Pluronic F68 would favor the mobility of the polysaccharide

molecules and facilitate the plasticizing effect by scCO₂. Besides, Pluronic F68 decreases interfacial tension between the water present in the membranes and CO₂ [17], aiding the diffusion of CO₂ in the membranes and their consequent plasticization.

The observed swelling was irreversible, probably because of the limited flexibility and elasticity of the produced material. The employed depressurization rate, which influences the polymers relaxation rate [18], might also have been a cause of the irreversible swelling.

3.2. Surface area, real density and porosity

Pore size and structure play an important role in the efficacy of skin scaffolds, which must have high porosity, with large interconnected pores, favoring cellular migration and reorganization, as well as the diffusion of nutrients and the removal of metabolic wastes. Furthermore, porosity regulates important processes for tissue regeneration such as angiogenesis and permits tissue growth and integration with the surrounding healthy tissue [19-21]. Surface area is also relevant because the greater the surface area, the greater the area to provide a suitable environment to which cells could adhere, migrate and grow.

According to Table 1, the real density of the non-processed samples decreased in the order P0%>P10%>P2%, as a consequence, the calculated porosity decreased in the order P10%>P0%>P2%. The surface area results followed the same tendency observed for real density.

It can be noticed that, despite the fact that membranes prepared with growing amounts of Pluronic F68 have increased pore sizes (as indicated by SEM analysis) and thickness, the measured real density and the calculated porosity did not followed the same tendency for sample P2%. It can also be noticed that sample P2% has the smallest

number of micropores, since N_2 adsorption analysis is sensitive only to pores smaller than $0.1\ \mu\text{m}$.

Regarding scCO_2 processed samples, it was noticed that real density and porosity tended to increase after scCO_2 processing (Table 1). It is believed that CO_2 caused a rearrangement of the polymeric molecules by interacting with some polysaccharides groups, such as $-\text{O}-$ and $-\text{NH}_2$ [22, 23], inducing the approximation of the polymeric chains. Consequently, the membranes' lamellae became more compact, as indicated by the increase of real density. On the other hand, surface area did not show a clear tendency of variation with pressure (Table 1).

The effect of Pluronic F68 on real density and porosity upon processing with scCO_2 is not evident at first. However, when the relative increases of real density and porosity are calculated, it can be noted that sample P2% presented the highest increase of porosity and sample P10% presented the highest increase of real density, which shows better plasticization by scCO_2 in the presence of Pluronic F68.

According to the literature, skin scaffolds should have porosity greater than 0.9 [1, 24]. Therefore, samples P10% processed at 100 and 300 bar would be more adequate for this use.

3.3. *Hydrophilicity analysis*

The behavior of membranes in the presence of moisture is another important characteristic to be evaluated. In the case of use in skin lesions, biomaterials should be capable of retaining moisture at appropriate levels, since dry dressings may desiccate the wound and too much moisture may result in maceration of the wound bed. Water vapor sorption, permeability and water contact angle results are shown in Figure 3.

Water vapor sorption capacities were measured during time, and the values shown in

Figure 3 represent the maximum values attained after saturation, which occurred around 8 hours. Water vapor sorption increased according to Pluronic F68 proportion, which is in accordance with the increase of thickness and changes in morphology. It was noted that scCO₂ processing at 300 bar significantly increased water vapor sorption capacity for all samples. The relative increases were 42%, 53%, and 41% for samples P0%, P2% and P10%, respectively. On the other hand, processing at 100 bar significantly increased water vapor sorption only for sample P10%, in 37%.

As previously mentioned, scCO₂ may have caused irreversible swelling of the polymeric network. Therefore, the processed samples would have more void spaces inside their structure, absorbing more moisture. The increase in water vapor sorption after supercritical processing was considered as a positive result, since it implicates in a better capacity of absorbing wound exudates and improved mass transfer inside the membrane.

In comparison with the literature, water vapor sorption capacities for N-carboxybutylchitosan and agarose wound dressings were of 30% to 50%. These values were considered adequate for commercial wound dressings [8]. Water vapor sorption capacities of wound dressings made of collagen and cellulose, N-carboxybutylchitosan and hyaluronic acid were reported to be 57%, 29% and 36%, respectively, and these values were also considered as convenient [25].

Therefore, according to these authors, water vapor sorption capacities from 29 to 57% may be considered as suitable. Given that P2% samples processed with scCO₂ at 300 bar as well as processed and non-processed P10% samples were able to attain values in this range, these biomaterials may be suitable for the treatment of skin lesions with this regard.

Water vapor permeability of the non-processed samples also increased with Pluronic

F68 proportion due to changes in morphology. The supercritical processing of samples P0% did not alter their permeability values significantly. However, permeability increased for samples P2% and P10% after processing at 100 bar. Further increases in permeability were not observed after processing at 300 bar.

It is recommended that the water vapor transmission rate for wound dressings is in the range between 300 to 3000 g/m².day, depending on the type of injury and on the healing phase[26]. In terms of permeability, it means that the obtained membranes should present values between 0.18 to 12.4x 10⁻¹⁴ kg.s⁻¹.m⁻¹.Pa⁻¹. Therefore, it can be concluded that the membranes would be more adequate for the treatment of wounds producing low amounts of exudate.

The measured water contact angles indicate that the membranes are hydrophilic, since values lower than 90° [27] were attained. The obtained results did not show a tendency of variation according to the Pluronic F68 proportion or scCO₂ pressure.

3.4. Simultaneous data thermal analysis (SDT)

The thermal behavior of the isolated polymers and the membranes were analyzed by thermal gravimetric analysis (TGA) and differential scanning calorimetry (DSC). The results obtained are shown in Figure 4. The main events observed in the thermal gravimetric analysis (TGA) are summarized in Table 2.

The TGA analysis of chitosan and alginate showed two thermal events. The first one, attributed to water loss, occurred at temperatures lower than 100°C. The subsequent thermal events were associated to the degradation of the polymers.

In the analysis of chitosan, a mass loss of 44% was verified in the second thermal event, with a peak at 300°C. Similar data were found by Martins *et al.*[28], who observed a degradation peak at 312°C with a mass loss of 59.8%. This degradation step can be

attributed to depolymerization and pyrolytic decomposition of the polysaccharide[29].

A mass loss of 33% was detected for the thermal degradation of alginate in the second event, with a peak at 244°C. The literature shows similar results regarding the degradation of alginate, with a peak at 226°C and a mass loss of 40%, which is attributed to the formation of Na_2CO_3 and carbonized material [30].

Differently from chitosan and alginate, Pluronic F68 had a single thermal event, with a degradation peak at 390°C, and a mass loss of 96%. Wu *et al.*[31] also observed that Pluronic F68 degraded in a single step, but at a lower temperature, of 320°C.

In the TGA analysis of the non-processed membranes, the first thermal event, which can be attributed to water loss, occurred at peak temperatures varying from 51 to 60°C, with mass losses varying from 12 to 21%. The more porous membranes had higher water losses, what may be explained by their highest water vapor sorption. The main degradation peaks of samples P2% and P10% occurred at 234 and 232°C, respectively, which are temperatures lower than the degradation temperature of pure alginate (244°C). On the other hand, sample P0% had a degradation peak at 275°C, which is a temperature lower than the degradation temperature of pure chitosan. A shoulder around 230°C was also observed, which is a temperature lower than the degradation temperature of pure alginate. The different thermogravimetric behaviors between non-processed samples can be due to the presence of Pluronic F68, which may alter the interactions involved in the formation of the polyelectrolyte complex (PEC).

The presence of a single degradation step may be considered as an indicative of the presence of a PEC according to Smith *et al.*[32], who observed that the chitosan-alginate complex had a degradation peak at 260°C. Anbinder *et al.*[33] also observed that alginate capsules covered with chitosan and crosslinked with calcium ions had a degradation peak at 250°C, with mass loss of 40%. Therefore, it can be inferred that the

formation of a chitosan and alginate PEC is favored in the presence of Pluronic F68.

Sample P10% exhibited also a small degradation peak at 393°C, which could indicate that the surfactant remains in the structure of the membranes even after successive washing steps. However, the residual presence of Pluronic F68 does not negatively affect the biocompatibility as detected by incubation of the material with L929 fibroblasts [5].

TGA analysis of scCO₂ processed membranes indicated significant changes in the behavior of sample P0%, which had its main degradation peak narrowed and shifted to a lower temperature. In addition, the shoulder at 230°C disappeared. These results may indicate that the PEC became more stable, since the presence of only one degradation peak is an indicative of PEC formation. On the other hand, the behaviors of samples P2% and P10% were not altered. It is believed that, since Pluronic F68 favors the formation of PEC, the chitosan-alginate interactions were already strong enough in these samples before scCO₂ processing.

The DSC analysis of chitosan and alginate independently showed two thermal events, as observed in the TGA analysis. The endothermic peaks at 75 and 79°C can be attributed to the vaporization of adsorbed water, corroborating the TGA curves. The exothermic peaks observed for these polymers also corroborate to the TGA curves and the literature[33]. Alginate had a degradation peak at 247°C and chitosan had a degradation peak at 310°C.

Pluronic F68 had two endothermic peaks in the DSC analysis, one at 56°C and other at 395°C. The first one is due to melting, according to similar data obtained by Verger *et al.*[34]. The second one can be related to oxidation or decomposition, since a degradation peak at 390°C was observed in the thermal gravimetric analysis.

In the DSC analysis of the non-processed membranes, the first endothermic peak was

attributed to water loss. Sample P0% had two exothermic peaks, at 217 and 290°C. On the other hand, samples P2% and P10% had only one exothermic peak, at 236 and 233°C, respectively.

It is believed that, in the analysis of sample P0%, the characteristic peaks of pure chitosan and alginate were shifted in -20°C and -30°C, respectively. On the other hand, samples P2% and P10% had only one degradation peak, at a temperature near the characteristic peak of pure alginate. According to Anbinder *et al.*[33], the shifting of the characteristic temperature of alginate could indicate the presence of a PEC. Unlike the TGA analysis, the peak related to Pluronic F68 could not be detected by DSC in samples P10%.

The DSC analysis of the processed samples indicated that only sample P0% was modified by scCO₂, changing from two to only one exothermic peak at a temperature lower than the characteristic peak temperature of alginate, which indicates the increase in chitosan-alginate interactions, as observed in the TGA analysis.

4. Conclusions

Chitosan-alginate membranes prepared in the absence or presence of different Pluronic F68 proportions, processed or not with scCO₂ at 45°C and 100 or 300 bar for 2 hours, presented adequate characteristics for the treatment of skin injuries with low amounts of exudate.

By increasing Pluronic F68 proportions, biomaterials with higher thickness, water vapor sorption and permeability were obtained. The presence of this surfactant during membrane preparation also resulted in improvements of chitosan-alginate interactions and facilitated the plasticization of the material by scCO₂. Supercritical CO₂ processing

caused the same effects as Pluronic F68, besides increasing the biomaterials porosity. Therefore, it is believed that scCO₂ and Pluronic F68 may play a similar role regarding structuring the chitosan-alginate PEC.

ScCO₂ processing of membranes prepared with Pluronic F68 resulted in materials with better prospects for use as wound dressings. Particularly, sample P10% processed with scCO₂ in both conditions employed would be also adequate for use as a skin scaffold in tissue engineering.

Therefore, the obtained results indicate high potential of supercritical technologies for processing chitosan-alginate PEC membranes, aiming at changes of their physicochemical properties or even for the impregnation of bioactive compounds.

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Figure Captions

Figure 1. High pressure cell with scCO₂ containing 1 cm x 1 cm membrane samples in stainless steel supports.

Figure 2. Scanning electron microscopy (SEM) of the prepared samples in the presence of different Pluronic F68 proportions, before and after supercritical CO₂ processing at 100 or 300 bar for two hours at 45°C (images magnifications was 150×, and for details 1500×).

Figure 3. Water vapor sorption (A), permeability (B) and water contact angles (C) of the prepared samples in the presence of different Pluronic F68 proportions, before and after supercritical CO₂ processing at 100 or 300 bar for two hours at 45°C and different pressures. Samples: P0% (□), P2% (■) and P10% (■).

Figure 4. TGA and DSC analysis of the porous chitosan-alginate membranes prepared in the presence of different Pluronic F68 proportions, before and after supercritical CO₂ processing and pure chitosan, alginate, Pluronic F68: **A-D** thermal gravimetric analysis and its derivative and **E-H** calorimetric analysis.

Biopolymers CO₂ processing

Table 1. Physical properties of chitosan-alginate based samples before and after supercritical CO₂ processing at 100 or 300 bar for two hours at 45°C.

Samples	Process conditions	Thickness, mm	Real density, g/cm ³	Porosity	Surface area, m ² /g BET Method
P0%	Non processed	0.12 ± 0.02 ^a	0.947 ± 0.013 ^{cd*}	0.546 ± 0.009 ^{bcd}	5.63 ^{e*}
	45°C, 100 bar	0.12 ± 0.02 ^a	1.302 ± 0.039 ^f	0.603 ± 0.030 ^{bd}	6.0 ± 0.1 ^e
	45°C, 300 bar	0.13 ± 0.01 ^a	1.366 ± 0.076 ^f	0.737 ± 0.020 ^e	3.4 ± 0.2 ^d
P2%	Non processed	0.36 ± 0.03 ^{bc}	0.252 ± 0.002 ^{a*}	0.227 ± 0.011 ^a	1.04 ^{ab*}
	45°C, 100 bar	0.63 ± 0.02 ^{cde}	0.230 ± 0.002 ^a	0.485 ± 0.020 ^{bc}	2.2 ± 0.1 ^{ac}
	45°C, 300 bar	0.59 ± 0.02 ^{cde}	0.287 ± 0.004 ^a	0.605 ± 0.012 ^{bd}	1.7 ± 0.1 ^{abc}
P10%	Non processed	0.50 ± 0.04 ^{bcd}	0.573 ± 0.003 ^{b*}	0.727 ± 0.004 ^e	7.67 ^{f*}
	45°C, 100 bar	0.71 ± 0.10 ^{def}	1.031 ± 0.029 ^{cde}	0.900 ± 0.004 ^f	2.0 ± 0.2 ^{abc}
	45°C, 300 bar	0.84 ± 0.06 ^{ef}	1.146 ± 0.033 ^{de}	0.914 ± 0.002 ^f	4.1 ± 0.2 ^d

Different letters in the same column indicate significant difference at 95% confidence limits (Tukey test). *Reference Bueno *et al.* 2014.

Table 2. Thermal events observed in the thermal gravimetric analysis of chitosan, alginate, Pluronic F68 and the chitosan-alginate samples before and after supercritical CO₂ processing at 100 or 300 bar for two hours at 45°C.

Samples		1 st event			2 nd event			3 rd event		
Pluronic F68, %		Range (°C)	Peak (°C)	Mass loss (%)	Range T(°C)	Peak (°C)	Mass loss (%)	Range T(°C)	Peak (°C)	Mass loss (%)
Components	Chitosan	25-180	59	11	180-358	300	44	-	-	-
	Alginate	25-180	62	18	180-288	244	33	-	-	-
	Pluronic F68	290-442	390	96	-	-	-	-	-	-
P0%	Non processed	25-180	57	12	180-400	275	40	-	-	-
	45°C, 100bar	25-180	55	15	180-400	230	42	-	-	-
	45°C, 300bar	25-180	53	13	180-400	241	41	-	-	-
P2%	Non processed	25-180	60	19	180-275	234	27	-	-	-
	45°C, 100bar	25-180	61	17	180-275	233	28	-	-	-
	45°C, 300bar	25-180	55	18	180-275	235	27	-	-	-
P10%	Non processed	25-180	51	21	180-275	232	25	355-430	393	6
	45°C, 100bar	25-180	67	18	180-275	231	25	355-430	390	6
	45°C, 300bar	25-180	55	17	180-275	233	25	355-430	393	7

FIG1

CO_2

CO_2

CO_2

Samples

Samples

FIG 4