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# 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 $CO_2$ ($scCO_2$) 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 h 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 $scCO_2$ according to thermal analysis. The results indicate that supercritical $CO_2$ processing made the membranes more adequate for use as wound dressings.



## 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 $CO_2$ ($scCO_2$) 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 $CO_2$ 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 $CO_2$ 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

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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 $CO_2$ 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 $scCO_2$ technology to process polymer matrices, and the lack of data regarding the use of this strategy, $CO_2$ 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 (capilar viscosimeter Ostwald–Cannon–Fenske, n.200), being equal to $1.26 \times 10^6$ g/mol for chitosan and $4.69 \times 10^4$ 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 $CO_2$ 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 thermosthatic bath Q-214M2, Quimis). The final suspension was homogenized at 1000 rpm for 10 min. Afterwards, 1 M NaOH was added to increase the pH to 5.3, under the same stirring rate, for 10 min. Then, 3.6 mL of 2% (w/v) $CaCl_2$ 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 min more. The amount of $CaCl_2$ 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 min 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 min 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 h, 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.

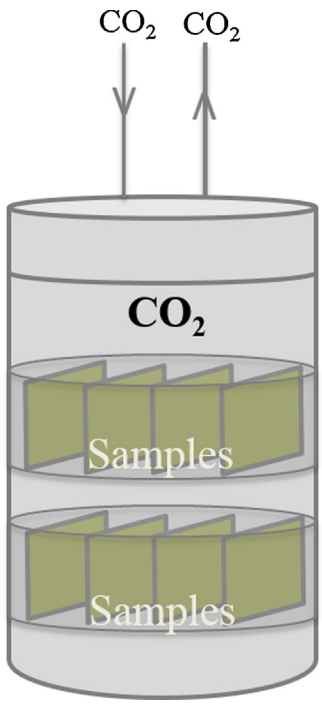


**Fig. 1.** High pressure cell with $scCO_2$ containing 1 cm × 1 cm membrane samples in stainless steel supports.

### 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 $cm^3$ of internal volume, a temperature-controlled water bath (Haake DC30, Thermo Electron Corporation) and a pressure transducer (Datum 2000™, Setra).

The cell, containing 1 cm × 1 cm membrane samples in stainless steel supports (as shown in Fig. 1), was pressurized at 100 or 300 bar at 45 °C for 2 h. 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 h 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 h 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 h, since water may interfere negatively in the results. Finally, before water vapor permeability tests, the samples were previously kept at 50% RH for