The Influence of Preparation Conditions on the Characteristics of Chitosan-Alginate Dressings for Skin Lesions

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ABSTRACT: In this work, the preparation and characterization of membranes obtained through chitosan and alginate coacervation and designed for use as wound dressings were evaluated. The influence of different stirring rates and rates of addition of chitosan solution to alginate solution on the final characteristics of the biomaterial was analyzed in detail, aiming at a simple and easily scalable membrane production protocol. The results show that membranes with dry thickness from 66 to 80 μm, wet thickness from 106 to 633 μm, tensile strength varying from 6.86 to 31.14 MPa, elongation at break from 3.97 to 8.42%, and maximum water uptake up to 19 g of water per gram of membrane and that are able to prevent the permeation of bacteria can be obtained in a fairly reproducible way by the procedure established. The membranes prepared at flow ratio of 40 mL/h and stirring equal to 100 rpm showed a high potential for use on highly exuding wounds. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 2703–2710, 2008

Key words: membranes; biomaterials; polyelectrolytes; hydrogels; mechanical properties; chitosan; alginate; wound dressing; coacervates

INTRODUCTION

The skin is considered the largest organ of the body and has many different functions, such as protection against deleterious environmental effects, temperature regulation, and prevention of dehydration, besides providing support to blood vessels and nerves.[1,2] Thus, skin lesions can alter distinct physiological functions, resulting in disorders of the organism.

Different polymeric membranes have been developed for the recovery of cutaneous lesions such as burns, wounds caused by chronic illnesses, or resulting from accidents. Particularly, films based on polysaccharides such as chitosan and alginate have frequently been studied for this purpose.[1,3–12]

Chitosan is a linear polysaccharide derived from chitin. It is composed of blocks of β(1-4)-2-acetamide-2-deoxy-βD-glucopyranose and 2-amino-2-deoxy-β-glucopyranose.[6] This biopolymer can accelerate wound healing, stimulate the migration of polymorphonuclear and of mononuclear cells, accelerate the reepithelialization of the skin, promote cell differentiation, stimulate cell proliferation and histoarchitectural tissue organization, as well as activate fibroblast migration and proliferation in the wound area.[1,5,7,13] Many of the chitosan properties rely on its cationic nature, which allows it to interact with negatively charged biomolecules such as anionic polysaccharides, proteins, and nucleic acids, many of which are found in the skin.[14]

Alginates, on the other hand, are composed of copolymers of α(1-4)-L-guluronic and β(1-4)-D-mannuronic acid.[6] They are natural polysaccharides obtained from seaweed, known to facilitate wound healing and epidermal regeneration.[8] When in contact with the lesion bed, alginate solutions form a gel that facilitates dressing removal, decreasing the pain and the trauma associated with the dressing change procedure. In addition, it provides a moist environment that accelerates tissue granulation and reepithelialization.[1]

Systems simultaneously composed of chitosan and alginate offer the advantages of both materials and can be tailored for several biomedical applications,

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such as wound dressing and drug delivery systems. According to Paul and Sharma,[1] skin injuries treated with chitosan-alginate membranes show a substantial decrease in the healing period and minimum scar formation when compared with the use of conventional covers.

Among the techniques described in the literature for the preparation of chitosan-alginate membranes, the one proposed by Yan et al.[10] and later adapted by Wang et al.[7,8] is of particular interest if the aim is large-scale production. This methodology involves the preproduction of coacervates from the raw materials under controlled conditions, which results in a suspension of fine fibers of chitosan and alginate. The formation of chitosan-alginate fibers is based on both the cationic nature of chitosan and the alginate anionic characteristic in solution.[1,6–8,10] Despite the main interactions normally involved in this process, electrostatic attraction between the chitosan amino groups and the alginate carboxyl groups, hydrogen bonds and hydrophobic interactions can also be involved.[10] The fibers obtained can be further reticulated with calcium ions and then unbound soluble molecules of chitosan and alginate can be removed by centrifugation and washing of the pellet. The final coacervate suspension is then dried, producing a flexible and coherent film.

For the production of homogenous chitosan-alginate membranes, the reaction rate should be controlled to allow a complete reaction between the polymers. The addition of an organic solvent of low polarity, such as acetone, to the chitosan solution induces the polymer to assume a less extended conformation, which aids in controlling the rate of reaction between the polyelectrolytes, resulting in more uniform coacervates.[10] Also, pH should be appropriately adjusted, mostly if calcium ions are added to the mixture before membrane drying to improve internal polymer reticulation. Factors such as membrane composition, polymer concentration, type of reticulation agent, drug incorporation, and drying and sterilization protocols can affect the final characteristics of chitosan-alginate membranes, namely morphology, transparency, degree of hydration, drug release, and mechanical and permeation properties.

Although the rates of stirring and of addition of chitosan to alginate solution are of fundamental importance in the formation of membranes based on coacervates, thorough studies on these issues have not been found in the consulted literature. In general, membrane production is performed in bench scale, and no specific values for flow rates or mixing rates are mentioned. Also, no details about the reaction vessel characteristics are provided. Altogether, these facts strongly contribute to reduce the reproducibility of the final product characteristics, an important issue related to biomaterials production in industrial scale. Therefore, the purpose of this work was to study in detail the preparation and characterization of chitosan-alginate membranes designed for wound dressing, aiming to establish a simple, reproducible and easily scalable membrane production process that would not involve the removal of unbound soluble molecules of chitosan and alginate prior to fiber reticulation with calcium ions.

MATERIALS AND METHODS

Materials

Chitosan-alginate membranes were produced using 85% deacetylated chitosan and low-viscosity sodium alginate from Sigma Chemical (St. Louis, MO). The water employed was distilled and deionized in a Millipore MilliQ system. All other reagents used were of analytical grade.

Membrane preparation

The membranes were prepared based on adaptations of the procedures described by Yan et al.[10] and Wang et al.[7,8] The volume and concentration of both alginate and chitosan solutions were adjusted to facilitate sample manipulation, since the original procedure resulted in thin brittle samples that tore easily when the coacervates were isolated from unreacted chitosan and alginate chains. Basically, 90 mL of chitosan solution at 0.5% (w/w) in aqueous acid acetic 2% (v/v) and acetone 1 : 1 (v/v) were added through an infusion pump (model 670T, Samtronic) at flow rates from 20 to 40 mL/h to 90 mL of aqueous alginate solution at 0.5% (w/v) at stirring rates varying from 100 to 500 rpm, employing a mechanical stirrer (model Q-251D2, Quimis) with a three tilted-blade propeller (diameter equal to 4 cm). All experiments were performed at 25°C in a stirred round-bottom jacketed glass tank with an internal diameter of 5 cm and a height of 13 cm.

The suspension obtained was homogenized for 10 min at 1000 rpm. The pH was adjusted to 5.28 with the addition of 8.4 mL of 1*M* NaOH, and the mixture was mixed again at 1000 rpm for 10 more minutes. Then 1.8 mL of 2% (w/v) $CaCl_2$ aqueous solution were added to produce the reticulation of alginate L-guluronic acid residues on adjacent chains not bound to chitosan. The mixture was deaerated for 90 min using a vacuum pump (model Q-355B, Quimis) to eliminate air bubbles, transferred to polystyrene Petri dishes (15 cm in diameter) and dried in an oven with air circulation (model 410, Nova Ética) at 37°C for 20 h. The dried membranes were immersed in 150 mL of 2% (w/v) $CaCl_2$ aqueous solution for 1 h for further reticulation and then twice

TABLE I
Properties of Membranes Prepared Under Different Operational Conditions, in Accordance with the two^2 Experimental Design Study

Stirring rate (rpm)	Flow rate (mL/h)	Dry membrane thickness (μm)	Wet membrane thickness (μm)	Maximum water uptake capacity (g/g)	Water drainage ability (kg/m^2 day)	Mass loss in water (%)	Tensile strength (MPa)	Elongation at break (%)
100	20	66 ± 4.50	155 ± 14.14	15.12 ± 1.20	12.22 ± 0.89	14.45 ± 1.96	6.86 ± 0.78	3.97 ± 1.18
500	20	72 ± 24.30	106 ± 13.87	12.42 ± 1.64	13.24 ± 0.94	11.30 ± 2.99	19.34 ± 4.94	4.02 ± 1.11
100	40	78 ± 17.76	633 ± 21.68	19.20 ± 0.61	13.56 ± 1.12	20.70 ± 3.63	27.70 ± 2.87	8.42 ± 2.82
500	40	73 ± 18.68	162 ± 8.37	11.12 ± 4.06	12.51 ± 0.36	8.64 ± 2.91	31.14 ± 5.54	6.23 ± 1.37
300	30	68 ± 14.68	151 ± 5.48	13.08 ± 1.55	13.61 ± 0.24	9.99 ± 1.88	13.65 ± 2.52	4.32 ± 0.84
300	30	76 ± 21.74	149 ± 9.62	15.30 ± 1.45	12.91 ± 0.36	16.34 ± 2.52	21.44 ± 3.21	5.48 ± 1.01
300	30	80 ± 17.56	161 ± 9.62	16.05 ± 2.42	14.34 ± 1.04	16.18 ± 2.19	11.79 ± 8.99	4.43 ± 0.98

in 200 mL of deionized water for 1 h and dried at room temperature.

Prior to use, the membranes were sterilized with ethylene oxide (EO) by exposure to Oxyfume-30 (30% EO and 70% carbon dioxide) for 8 h at 40°C and a relative humidity of 40 to 50% at Acecil Central de Esterilização Comércio Indústria Ltda (Campinas, SP, Brazil), a specialized EO sterilization company. An initial vacuum of 400 mmHg was used for 15 min and Oxyfume-30 (600 mg/L) was added until the chamber reached a pressure of 0.5 kgf/cm^2. After the incubation period, the vacuum was reestablished (400 mmHg) and the samples were aired three times with nitrogen (N_2) for removal of residual EO from the membranes.[15]

A two-level factorial design with three central point replicates was used to investigate the effects of the two selected independent variables (stirring rate and flow rate of addition of chitosan to alginate) on dry membrane thickness, on responses related to the behavior of the membrane when exposed to water (wet membrane thickness, maximum water uptake capacity, percentage of mass loss, and water drainage ability) and also on membrane mechanical properties (tensile strength and strain at break). The design matrix is shown in Table I. The results were analyzed using the software Statistica 5.1 (StatSoft). Experimental data were expressed as mean ± standard deviation and significance levels (p) lower than 0.10 were assumed to be statistically significant in this study.

Membrane characterization

The thickness of dried and wet membranes (hydrated for 24 h with deionized water at 37°C) was measured at five different positions close to the membrane border and at 90° angles from each other and the results were expressed as averages. Measurements were obtained using a micrometer (Digimess).

The maximum water uptake capacity was evaluated by immersing dried 1 × 6 cm^2 samples with known weights (W_{dry}) in 5 mL of deionized water at 37°C. The weights of the wet samples (W_{wet}) were determined after 24 h, and the maximum water uptake capacities (C_w) were calculated with eq. (1). Excess water on membrane surfaces was removed by gently pressing the samples between two sheets of absorbent paper for 10 s prior to the weight measurement.

$$C_w = \frac{W_{wet} - W_{dry}}{W_{dry}} \quad (1)$$

Similarly, the percentages of membrane mass loss were determined by immersing dried 1 × 6 cm^2 samples with known weights ($W_{initial}$) in distilled water at 5°C for 43 days. After this period, the samples were dried at 37°C for 24 h and the weight of the samples (W_{final}) was determined. The mass loss (M) in water was calculated in accordance with eq. (2).

$$M(\%) = \left(\frac{W_{initial} - W_{final}}{W_{initial}}\right) \times 100 \quad (2)$$

The water drainage ability of the membranes was examined for samples (2.0 cm in diameter) previously hydrated with deionized water at 37°C for 2 h. The samples were placed on the surface of plastic flasks containing 7 mL of deionized water. The flasks were closed with plastic lids having circular holes of 1.2 cm in diameter. Rubber *o*-rings were used to avoid water leakage. The flasks were inverted and their bases were perforated with needles to equalize the pressure. The samples were incubated in a desiccator containing silica gel for 72 h at 37°C. Water drainage ability was estimated with eq. (3):

$$D = \frac{W_d}{A \times t} \quad (3)$$

where D is the drainage ability, W_d is the amount of drained water, A is the membrane area exposed to the dry air, and t is the period length.

The membrane tensile mechanical properties (five 1×10 mm^2 independent test samples per membrane type) were analyzed with a universal testing machine (model H5K-S, Tinius Olsen), employing a cell load of 20 kgf, a gauge length of 45 mm, and a crosshead speed of 10 mm/min.

In addition to all the membrane properties considered as responses in the factorial design analysis, *in vitro* protection against bacterial permeation and bacterial growth inhibition activity were also evaluated for all the membranes prepared. Also, selected samples were characterized for superficial surface and cross section morphology.

For evaluation of the *in vitro* protection against bacterial permeation, the samples (2.5×2.5 cm^2) were partially hydrated in sterile water for 30 s and placed on the surface of Petri dishes (90 cm in diameter) containing 20 to 25 mL of sterile Tryptic Soy Agar medium (TSA, Difco^{TM}) dissolved in distilled water (40 g/L). Aliquots of 100 μL of bacteria suspension containing either 5.0×10^8 Pseudomonas aeruginosa colony-forming units (CFU) per mL or 4.3×10^8 Staphylococcus aureus CFU/mL were added to each membrane sample. The dishes were incubated (incubator model 002-CB, Fanem) at 35°C ± 2°C for 48 h, when cell growth below the membrane surface was evaluated. The formation of a growth inhibition zone by membranes was evaluated by placing 1.5×1.5 cm^2 samples on the surface of solid TSA medium (40 g/L in distilled water), previously inoculated with 5.0×10^8 Pseudomonas aeruginosa CFU/mL or 4.3×10^8 Staphylococcus aureus CFU/mL. After incubation at 35°C ± 2°C for 48 h, the formation of clear zones surrounding the membranes indicating bacterial growth inhibition was assessed.

The morphology of the membranes was evaluated using a scanning electronic microscope (model Leo 440i, Leica) coupled to the Leo UIF series 400 software. The samples (5×5 mm^2) were lyophilized, placed on appropriate stubs, and sputtered-coated with an ultra-thin layer of gold (92 Å) in a coating apparatus (SC 7620 mini-sputter coater).

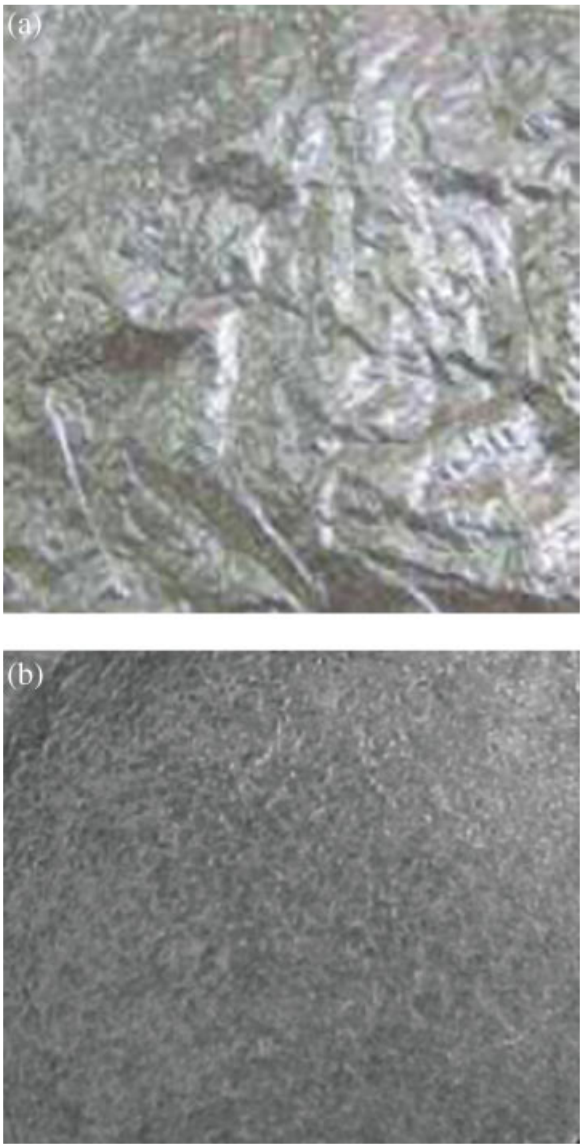

Figure 1 (a) Typical visual aspect of dry and (b) wet membranes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

RESULTS AND DISCUSSION

Effects of operational conditions on membrane behavior when exposed to water

The study of a simple and easily scalable procedure for preparation of chitosan-alginate membranes from the coacervation of both polymers resulted in membranes in dry and wet state having the typical visual aspects depicted in Figure 1 and the average properties described in Table I.

When in dry state, the membranes had irregular surfaces [Fig. 1(a)], while wet membranes were smooth [Fig. 1(b)], regardless of the operational conditions employed, similarly to previously reported data.[10] The membranes had no apparent pores on their surfaces, as also observed by Wang et al.[7] The membranes were reasonably transparent in either dry or wet state, which potentially allows effective observation of the wound bed during skin recovery.

The paretto charts of effects for dry membrane thickness, wet membrane thickness, maximum water uptake capacity, mass loss, and water drainage ability are shown in Figure 2. This figure also shows the paretto charts of effects for the responses tensile strength and elongation break, which will be discussed in Influence of Process Conditions on the Mechanical Properties of the Membranes section. The dashed line in each chart indicates the magnitude an

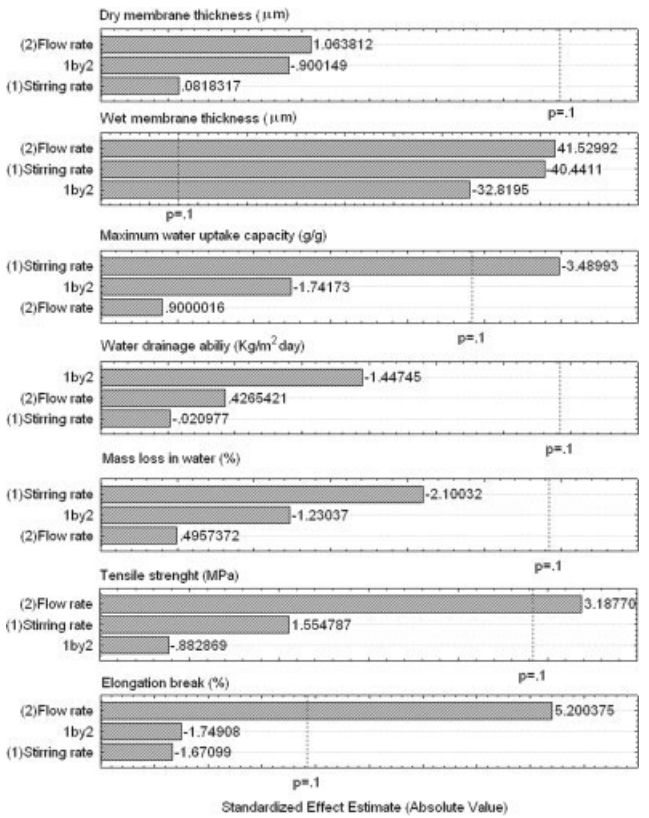

Figure 2 Paretto charts of effects showing the standardized effects of stirring rate and chitosan solution flow ratio on membrane dry thickness, wet thickness, maximum water uptake capacity, water drainage ability, mass loss when exposed to water for 43 days, tensile strength, and elongation break. Dashed line in each chart indicates the magnitude an effect should have to be considered statistically significant with a confidence level of 90%.

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It can be observed in Figure 2 that the effects of flow rate and stirring rate on wet thickness as well as their interaction were significant. In general, an increase in flow rate and a decrease in stirring rate increased the wet thickness. Also, agitation intensity had a significant effect on the maximum water uptake capacity, with low agitation resulting in increases in the response. The other effects were not significant at the confidence level considered.

When in dry state, membrane thicknesses were not significantly affected by the variation in the operational conditions used during the preparation procedure, ranging from 65 to 80 μm. These values are higher than the ones reported by Yan et al.[10] and Wang et al.,[7,8] which varied from 17.6 to 48.2 μm, probably because the volume of the polymeric solutions used in this work and their concentrations were higher than those employed by the above-mentioned authors.

Since the design purpose of an artificial dermis substitute is to construct a scaffold for the attachment and growth of skin fibroblasts rather than a permanent implant, dermis polymeric substitutes should be thinner than the human dermis, whose thickness varies from 0.5 to 2 mm depending on age, sex, and body area.[16] Considering this information, all membranes prepared in this work could be potentially employed as wound dressings.

After hydration, membrane thicknesses increased around twice for all preparations. The exception was the membrane prepared at 100 rpm and a flow rate of 40 mL/h, in which the thickness increased around eight times. These results are compatible with the maximum water uptake data, which show that water absorption by this membrane was significantly higher than those verified for the remaining preparations.

As shown in Table I, all membranes had high water uptake capabilities, varying from 11 to 19 g H_2O/g membrane, behaving as effective water absorbers. This is in agreement with previously reported results, of up to 16 g H_2O/g membrane.[10] Since the pH of the water used in the present work was around 5, and considering that the water uptake profiles for PEC membranes reported by Wang et al.[7,8] were pH-dependent, protonation followed by the repelling action of residual amino groups in the PEC films potentially caused film expansion, which improved water absorption.[10]

As discussed by Hoffman,[17] when a dry hydrogel begins to absorb water, the first water molecules to enter the matrix, referred to as primary bond water, hydrate the most polar hydrophilic groups. As a result, the polymeric network swells and exposes the hydrophobic groups, which forces the reorganization of water molecules, leading to a secondary hydration process. After that, the polymeric network interstices soak up additional water molecules, and these additional water molecules, incorporated after the ionic groups become saturated, are called free water or bulk water.[17] As a consequence of the membrane preparation procedure employed in the present work, probably only the free water was removed from the membranes with absorbing paper, while the incorporated water still associated with the polymeric network was responsible for the high water uptake capacities observed.

The membranes which had the highest and lowest water uptake capacity, prepared with 40 mL/h and mechanical stirring of 100 and 500 rpm, respectively, were further characterized by scanning electron microscopy. Both membranes had irregular surfaces with small elevations distributed throughout with no apparent pores (Fig. 3). However, the cross-sectional morphological analysis showed significant differences in fiber compaction (Fig. 4), which may have

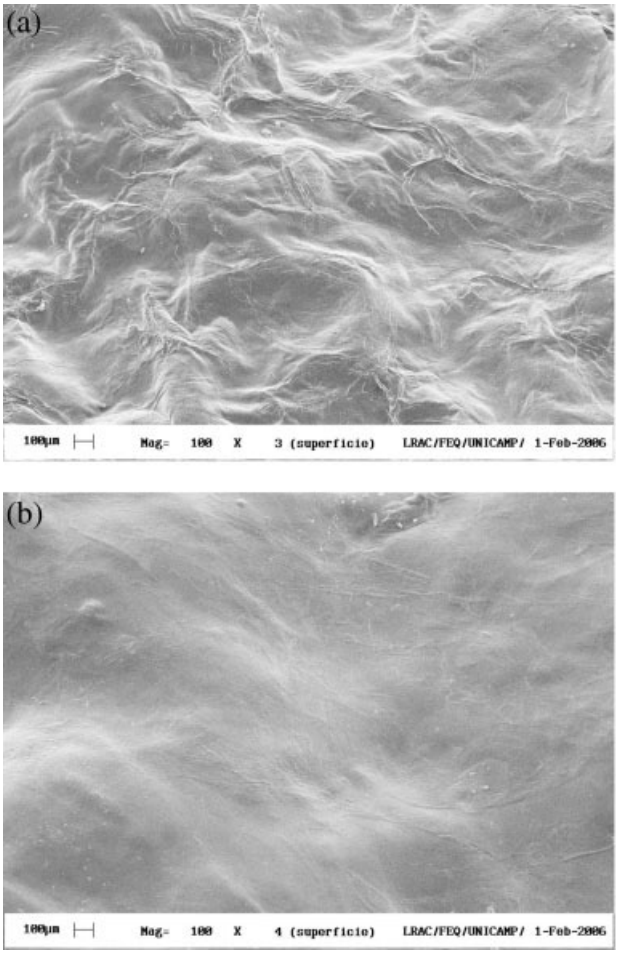

Figure 3 Surface of the membranes (×100) at a 40° angle, prepared respectively, at flow ratios and stirring rates equal to (a) 40 mL/h and 100 rpm and (b) 40 mL/h and 500 rpm.

caused the differences in the water uptake capacities of the observed membranes. The chitosan-alginate complexes obtained at low stirring rates probably form longer fibers and, consequently, less compacted membranes.

Aiming to evaluate membrane efficiency regarding to exudate permeation, the membranes were placed in direct contact with water to simulate wet lesion conditions. The results obtained for water drainage ability indicated in Table I show that all membranes prepared had high water drainage abilities, with values of around 13 kg/m^2 day. As pointed out by Behar et al.,[18] the average natural body liquid loss through normal skin is around 0.240 kg/m^2 day, while damaged surfaces have a higher liquid flow, varying between 3.4 and 5.2 kg/m^2 day, depending on the type of the lesion. Since permeability values of around 5 kg/m^2 day can be considered adequate for dressings to be used on lesions with high exudate production,[18] all membranes prepared can potentially be used on these kinds of wounds.

After exposure to water for 43 days, the membranes showed relatively low mean mass losses, varying from 8 to 20%. The membrane that had the lowest mass loss also had the lowest water uptake capacity, suggesting that these characteristics are closely related. The mixing of chitosan and sodium alginate solutions gives rise to a suspension of fine fibrous chitosan-alginate coacervates, also containing unreacted chitosan and alginate molecules, which in accordance with the procedure adopted, were not removed. Since the complex formed between chitosan and alginate is insoluble in water,[10] the mass loss verified in this work may probably be attributed to unreacted alginate molecules, suggesting that during storage, these molecules become soluble in water with a mass loss proportional to the membrane water uptake capacity.

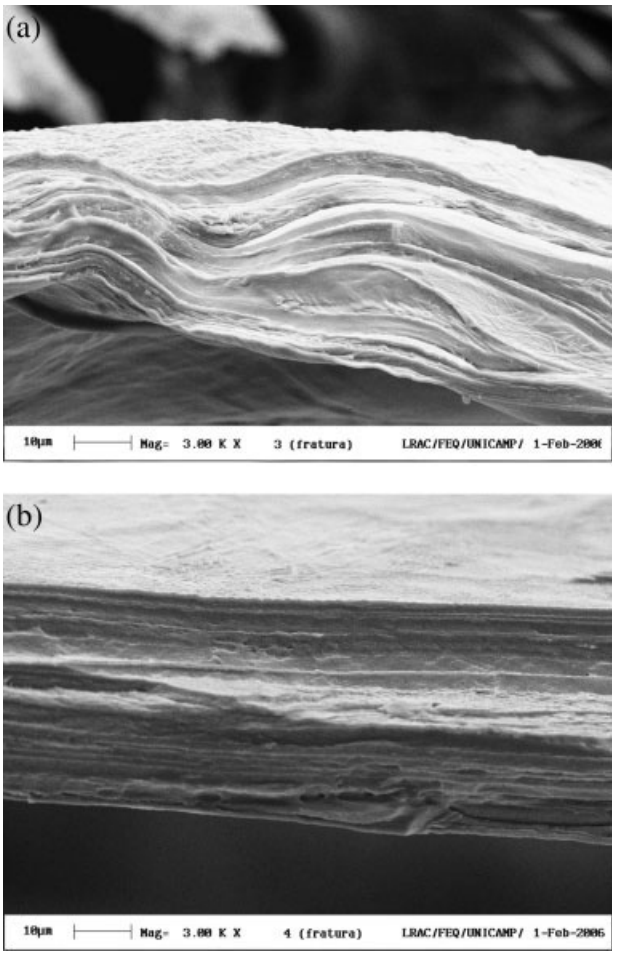

Figure 4 Cross section of the membranes (×3000) prepared respectively, at flow ratios and stirring rates equal to (a) 40 mL/h and 100 rpm and (b) 40 mL/h and 500 rpm.

The membranes prepared at a flow rate of 40 mL/h and mechanical stirring of 100 rpm had low fiber compaction, high water uptake capacity, and high mass loss, while the ones prepared at the same flow rate but with a stirring rate of 500 rpm had high fiber compaction, low water uptake, and mass loss. In addition, these dry membranes showed to be around 13% denser than the membranes prepared at 100 rpm, suggesting that their compact structure promotes a reduction in water uptake capacity, hampering water penetration in the system, indicating indeed a quantitative relationship between membrane mass loss and membrane water uptake capacity.

Influence of process conditions on the mechanical properties of the membranes

The values obtained for the mechanical properties of the membranes are shown in the two last columns in Table I. In Figure 2 the paretto charts of effects for tensile strength and strain at break are shown. It can be observed that an increase in the stirring rate increases tensile strength and decreases elongation at break. At the confidence level considered, however, the only significant factor was flow rate, which positively affected the two responses.

The tensile strength values obtained varied from 6 to 31 MPa, which can be considered adequate for membranes used for wound dressings, since the tensile strength for normal skin ranges from around 2.5 to 16 MPa.[8] The results obtained showed a substantial improvement in tensile strength in comparison with the previously reported data, which varied from 4.49 to 18.72 MPa for similar membrane preparation procedures, although including different membrane processing steps.[7,8,10]

Membranes prepared at lower flow rates, due to the longer period used in their preparation, had a more accentuated increase in viscosity, due to increased acetone evaporation during the process. Therefore, the use of low stirring rates may have hampered the mixture homogenization, affecting the formation and homogenization of the coacervates, and consequently reducing membrane tensile strength.

The average values of strain at break observed for the membranes produced, always below 10%, can be considered low in comparison with that of normal skin, which has an elongation at break of around 70%.[19] These results are similar to those previously reported for this kind of membrane.[7,8,10] However, this property was evaluated for dry membranes, a condition potentially different from that of final application. As previously shown, the membranes produced can absorb from around 11 to 19 times their weight in water, which can act as a plasticizer when imbibed in the membrane matrix, significantly increasing the strain at break.

Membrane ability to protect against bacterial permeation and growth

Skin lesions, such as some types of burns, can have devitalized and humid tissues, providing ideal conditions for microbial proliferation. When these lesions are not protected, they can be colonized by bacteria within 12 to 24 h and in 48 h, around 100 million microbes can be found per gram of skin.[9] Infections are one of the most frequent causes of death among burn patients, due to their vulnerability to microorganism invasion until the skin has been completely regenerated.[9] Therefore, wound dressings capable of protecting the lesions against microbial attack are highly desirable.

Since the protection against bacterial permeation and proliferation provided by chitosan membranes when associated with alginate can be attributed to the fibrous structure of the samples, which hinders cell migration through the membrane, and to the antibacterial properties of the chitosan itself,[4] both hypotheses were tested using microorganisms typically found in skin lesions in hospitals.

The results achieved for all chitosan-alginate obtained, regardless of the operational conditions employed, indicated that despite the fact that no cell growth inhibition zone was formed by any of the samples, the membranes can be potentially used as wound dressings, since no bacterial permeation was observed. This behavior suggests that after association with alginate, chitosan loses its bactericide activity (probably due to the blockage of chitosan free amino groups by alginate carboxyl groups), evidenced by the abscence of inhibition zones when the membranes were placed on the surface of previously inoculated media. Regardless of the loss of bactericide activity, when inoculated on membrane surfaces, growth of both Pseudomonas aeruginosa and *Staphylococcus aureus* was limited to the area where they were applied. Swabs from the bottom part of the membranes in direct contact with the culture media did not result in cell growth.

CONCLUSIONS

The results achieved show that through the use of a simple and easily scalable membrane production protocol involving controlled process operational conditions membranes with reproducible characteristics can be successfully obtained. Employing stirring rates from 100 to 500 rpm and chitosan flow ratios from 20 to 40 mL/h, it is possible to obtain coherent and uniform fibrous membranes with adequate thickness, high water drainage ability, appropriate

tensile strength, and high stability in water, meeting the necessary requirements for use as wound dressings. The membranes obtained using a flow rate of 40 mL/h and a mechanical stirring of 100 rpm had the most satisfactory overall results, showing the highest potential for use as dressings of skin lesions containing large amounts of exudates. In these wounds, a dressing with high liquid absorption capacity would be recommended to maintain adequate moisture level in the wound bed, while draining the excess of liquid that could delay lesion recovery. The mass loss of around 20% in 43 days would not consist in a problem because, this mass loss can be considered low, since probably the dressing would not be in contact with the lesion for such a long period.

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