Study of the Swelling and Stability Properties of Chitosan–Xanthan Membranes

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ABSTRACT: The production of stable membranes formed by chitosan–xanthan complexation employing different polysaccharide concentrations and chitosan flow rates is described in this work. The membranes were characterized in terms of their morphology, thickness, maximum uptake capacity, and mass loss in physiological solutions, water drainage ability, and mechanical resistance. The results obtained show that it is possible to produce membranes with high swelling capacities and drainage ability (up to 61 g of H_2O per gram of dry membrane and 5900 g m^{-2} day^{-1}, respectively) without significant mass loss when exposed to aqueous solutions (maximum 13%). The membranes obtained at the chitosan solution flow rate of 300 mL h^{-1} had the most suitable mechanical properties. Increases in polysaccharide concentration produced higher water absorption and reduction in tensile strength at break. Therefore, the material obtained showed properties that suggest its promising applications as wound dressings or as scaffolds for animal cell cultures. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: E154–E160, 2012

Key words: membranes; biological applications of polymers; biomaterials; chitosan; xanthan

INTRODUCTION

Polyelectrolyte complexes (PECs) are commonly formed by the interaction of oppositely charged polymers, such as chitosan and xanthan, through electrostatic attraction forces. Secondary interactions, such as hydrogen bonds, hydrophobic interactions, and Van der Waals forces, can also occur when polysaccharides are involved, contributing to stabilize the structure formed. These interactions may lead to changes in the material properties,[1,2] affecting, for instance, pH stability and water absorption. During complexation, the polyelectrolytes may form coacervates or macromolecular networks that can easily swell in biological fluids.[3]

Chitosan is a cationic polysaccharide derived from chitin, the second most abundant natural organic material, found in the exoskeleton of crustaceans, mollusks and insects as well as in the cell wall of some fungi.[4] This compound has interesting biological properties such as high biocompatibility, biodegradability, and bioactivity,[5,6] and is able to form stable complexes with negatively charged molecules, such as alginate and xanthan. Because of these characteristics, chitosan has several applications in the biomaterials area.

Xanthan is a heteropolysaccharide obtained through fermentation by *Xanthomonas campestris* frequently used as a rheology modifier in pharmaceutical, cosmetic, and food industries. It is a nontoxic anionic polymer that is stable over wide ranges of temperature and pH and has a good texture and a high liquid absorption capacity.[7,8] The majority of the reported studies on chitosan–xanthan complexes focus on the production of gels, tablet matrixes or particles aiming at the encapsulation of cells, enzymes, and also active agents.[9–18] To our knowledge, no detailed reports on the investigation of these complexes for the formation of membranes are currently available.

Membranes formed by chitosan–xanthan complexation have potential to be employed in many different areas, e.g., as wound dressings, drug delivery devices, scaffolds in tissue engineering, and even biodegradable films for food coating. For these purposes, the membranes should show adequate swelling and stability in aqueous solutions as well as satisfactory permeability to chosen solutes, among other relevant properties.

The aim of the present work was to develop stable membranes composed of different ratios of chitosan to xanthan and to investigate their properties, mostly in relation to swelling behavior in aqueous solutions.

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EXPERIMENTAL

Material

The membranes were produced using 98% deacetylated chitosan (Ch) from crab shells (Sigma-Aldrich, USA), acetic acid (Synth, Brazil) and Keltrol® F xanthan (X) (CPKelco Brazil S/A, Brazil). 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 the procedure described by Rodrigues et al.[19] and adapted for the use of xanthan as a polyanion instead of alginate, with the advantage of not requiring the addition of acetone or calcium chloride in the mixture to improve membrane coherence. Briefly, chitosan 0.5 or 0.75% (w/v) aqueous solutions were prepared in 2% acetic acid, while xanthan was dissolved directly in water at concentrations of 0.25, 0.5, and 0.75% w/v. Each membrane formulation was obtained by the addition with an infusion pump (model 670T, Samtronic) of 90 mL of chitosan solution (at flow rates from 40 to 300 mL h^{-1}) to 90 mL of xanthan solution placed in a glass tank at 25°C under stirring at 1000 rpm with a three tilted-blade propeller coupled to a mechanical stirrer (model Q-251D2, Quimis). The polymeric mixture was then degassed with a vacuum pump (model Q-355B, Quimis) for 2 h, transferred to a polystyrene Petri dish (15 cm in diameter) and dried in an oven with air circulation (model 410, Nova Etica) at 37°C for 24 h. The dried membranes were immersed twice in 200 mL of deionized water for 1 h and dried again at 37°C for 24 h. Then the membranes were sterilized with ethylene oxide by exposure to Oxyfume-30 (30% ethylene oxide and 70% carbon dioxide) for 8 h at 40°C and a relative humidity of 40 to 50% at Acecil Central de Esterilização Comércio e Indústria (Campinas, SP, Brazil).

Membrane characterization

The membranes were characterized in terms of their thickness, morphology, water drainage ability, uptake capacity, and mass loss in different aqueous solutions and also of their mechanical properties as follows (basically as suggested by Rodrigues et al.19).

Membrane thickness was measured at seven different positions using a micrometer (Digimess).

Membrane surface and cross-section morphology were evaluated using a scanning electron microscope (model Leo 440i, Leica). The samples (2 cm × 1 cm), previously stored for 24 h in a desiccator in the presence of activated silica, were criofractured with liquid nitrogen, placed on appropriate stubs and metalized through the deposition of a 92 Å gold layer (mini Sputter coater, SC 7620).

The water drainage ability of samples (2 cm in diameter) previously hydrated with water at 37°C for 1 h was evaluated. The samples were placed on the top of conical plastic flasks containing 10 mL of deionized water. The flasks were sealed with rubber o-rings and closed with plastic lids having circular holes 1.2 cm in diameter. The flasks were inverted and their bases were perforated to equalize the pressure. The samples were then incubated for 72 h at 37°C in a desiccator containing silica gel and water drainage ability was calculated using eq. (1):

$$D = \frac{W_d}{A \times t} \tag{1}$$

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

The membrane hydration behavior (2 cm × 1 cm samples of known weights immersed in 5 mL of water) was monitored during 24 h at 37°C and the degree of swelling at different periods was calculated using eq. (2):

$$C_w = \frac{W_{\text{wet}} - W_{\text{dry}}}{W_{\text{dry}}} \tag{2}$$

where C_w is the water uptake capacity, W_{dry} is the initial weight of dry samples and W_{wet} is the weight of wet samples for each period evaluated.

The maximum daily uptake capacity of different solutions was similarly assessed by immersing dry samples (6 cm × 1 cm) of known weights in 5 mL of deionized water, saline solution 0.9% (SS), fetal bovine serum (FBS), or simulated body fluid (SBF) at 37°C. The weights of the wet samples were determined after 24 h and solution uptake capacities were calculated, also according to eq. (2).

The mass loss of membranes (6 cm × 1 cm samples) stored at 10°C for 40 days in the solutions described above was evaluated after drying the samples for 24 h at 37°C and storing them for 24 h in with silica gel. The mass loss was calculated through eq. (3):

$$M(\%) = \frac{W_{\text{initial}} - W_{\text{final}}}{W_{\text{initial}}} \times 100 \tag{3}$$

where M is the mass loss, W_{initial} refers to the initial sample weight and W_{final}, to the final sample weight.

The tensile mechanical properties of the membranes were evaluated using five independent test samples for each formulation (10 cm × 1 cm samples) in a universal testing machine (model H5K-S, Tinius

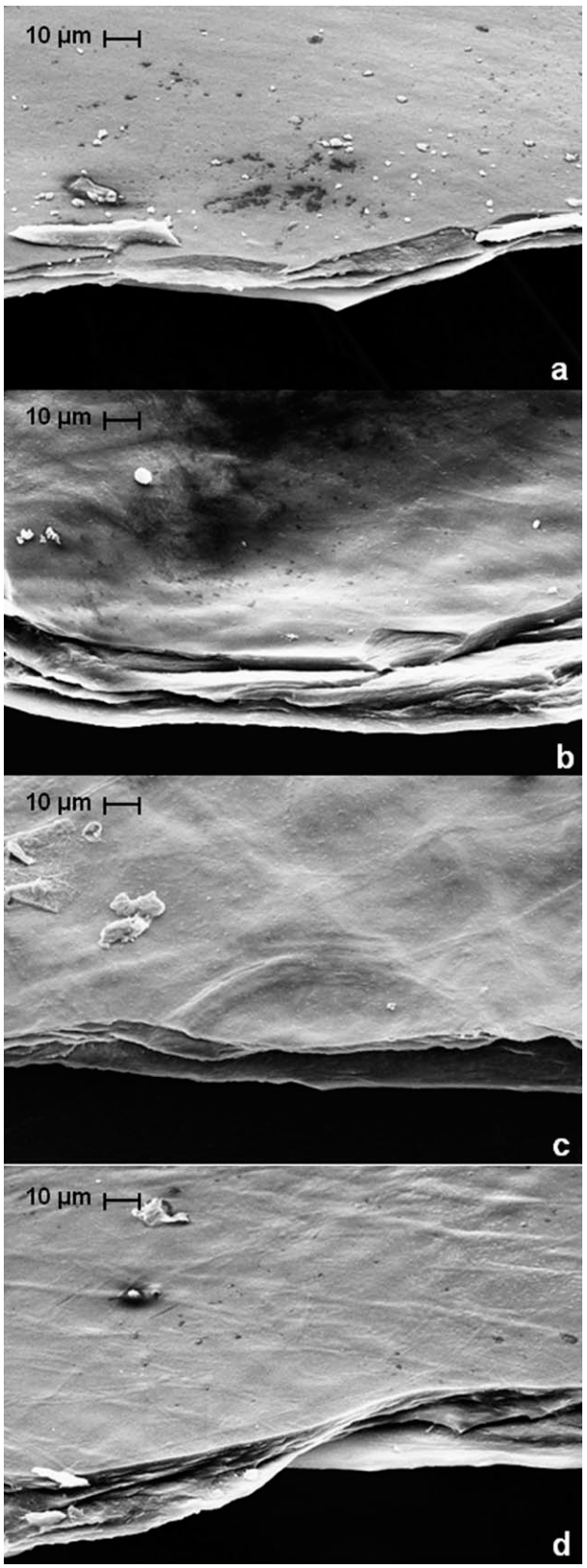

Figure 1 Membranes cross-section for different flow rates of chitosan solution addition: (a) 80 mL h^{-1}; (b) 120 mL h^{-1}; (c) 200 mL h^{-1}, and (d) 300 mL h^{-1}.

Olsen), employing a cell load of 20 kgf, a gauge length of 45 mm and a crosshead speed of 10 mm min^{-1}.

RESULTS AND DISCUSSION

Effects of the variation in chitosan solution flow rate on membrane properties

The flow rate of the addition of 0.5% chitosan solution to 0.5% xanthan solution was varied from 40 to 300 mL h^{-1}, and the effects on membrane characteristics were analyzed. High addition rates would be better in terms of duration of the membrane production process; however, low values would hypothetically allow improved reaction between the polysaccharides, resulting in more resistant membranes.

Interestingly, the use of a flow rate of 40 mL h^{-1} resulted in fragile membranes, which fragmented and/or dissolved during the characterization procedures. This behavior is a consequence of the long exposure time of the chitosan–xanthan complex to the intense shear stress associated with the mechanical mixing of the resulting material, which probably contributed to the disruption of the entangled fibers formed. The results attained for the membranes prepared under the remaining conditions are indicated in Figures 1 and 2 and Tables I–III.

All samples show a clear lamellar structure without perpendicular pores crossing their sides (Fig. 1), and a tendency towards reduction in membrane thickness was noted when the chitosan solution flow rate was increased (Table I), mostly from 120 to 300 mL h^{-1}, probably because larger fibers were obtained at the lower chitosan feeding rates. At the 80 mL h^{-1} flow rate, some chitosan–xanthan fiber shattering probably occurred, similarly to what was noted for the samples prepared at 40 mL h^{-1}, making the membrane surface smoother.

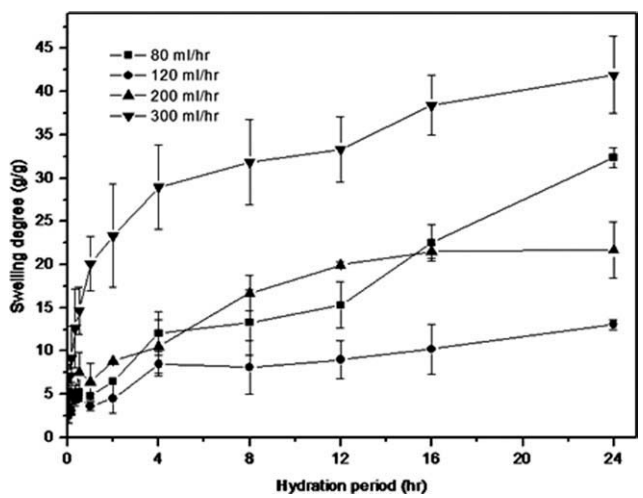

Figure 2 Hydration profile of chitosan–xanthan membranes prepared at different flow rates of chitosan solution addition.

TABLE I
Thickness, Water Drainage Ability, Tensile Strength, and Elongation at Break of Membranes Prepared Using Different Chitosan Solution Flow Rates

Flow rate (mL h^{-1})	Membrane thickness (μm)	Water drainage ability (g m^{-2} day^{-1})	Tensile strength (MPa)	Elongation at break (%)
80	164.29 ± 35.24[a]	3354.63 ± 189.75[c,d]	2.24 ± 1.14[f]	2.54 ± 0.93[h]
120	165.00 ± 33.22[a]	3180.00 ± 208.85[c]	4.74 ± 0.95[g]	1.60 ± 0.60[h,i]
200	122.86 ± 30.99[b]	3482.84 ± 158.11[d]	5.11 ± 1.33[g]	1.20 ± 0.71[i]
300	111.90 ± 25.74[b]	3967.23 ± 134.30[e]	5.23 ± 1.07[g]	1.82 ± 0.78[h,i]

Different letters indicate significant difference at 95% confidence limits (Tukey test).

There was a tendency for water drainage ability (Table I) to increase with chitosan solution flow rate in samples prepared with chitosan flow rates above 120 mL h^{-1}. Samples obtained at the flow rate of 80 mL h^{-1} showed increased permeability to water, probably due to fiber loss during solvent flow.

The evaluation of mechanical properties showed that the tensile strength varied from 2.24 to 5.23 MPa, while the elongation at break varied between 1.20 and 2.54%. The membranes obtained at higher flow rates showed the best results for tensile strength and the membranes produced at a chitosan solution flow rate of 80 mL h^{-1} showed the highest elongation at break. These results could potentially be attributed to different forms of polymeric fiber packing and also to their size. It is probable that at the flow rate of 80 mL h^{-1} smaller fibers were obtained, while at 300 mL h^{-1}, longer and better dispersed aggregates were achieved.

All membranes prepared had a high water uptake capacity (Table II), varying from 15.95 to 39.40 g H_2O/g dry membrane. These values are higher than those reported by Rodrigues et al.[19] for chitosan–alginate membranes, which varied from 11.1 to 19.2 g H_2O/g dry membrane. In saline solution, the membranes obtained using the highest flow rate absorbed around twice the amount absorbed by the membranes obtained at the two lowest flow rates. A similar trend was observed for the membranes exposed to SBF and FBS. In general, salt-containing solutions were less readily absorbed than water, probably because less free water was available.

Hydration of the chitosan–xanthan membranes in water was further studied and absorption was monitored during 24 h, as shown in Figure 2. The results show that water absorption occurred throughout the entire exposure period; however, the highest rates were observed during the first few hours of contact, increasing again at around 8 h of contact in most of the cases. It can also be observed that the water absorption increased with the chitosan addition flow rate, except for the samples prepared at 80 mL h^{-1}, which absorbed an intermediate amount of solvent. Because this particular preparation was exposed to the high shear stress of the mixing procedure for longer periods due to the lower rate of chitosan addition, the size of the coacervates formed might have been smaller and looser in this situation, thereby facilitating access of the water molecules through the membrane structure, which is corroborated by the data in Tables I–III. The expansion of the polymeric chains due to water incorporation during the first moments was followed by changes in the slopes of the absorption curves after 8 h of exposure to water, which may have been a result of partial xanthan dissolution. According to Bernabé et al.,[20] the integrity of chitosan–pectin polyelectrolyte complexes during water absorption is maintained by the reticulation of the chains. However, at either acidic or basic pH, ionic bonds can be disrupted, which was potentially the case noted here, since the pH of deionized water used was around 5.1. Rodrigues et al.[19] verified a similar behavior for chitosan–alginate membranes, attributing the increase in water absorption after 12 h to the discreet protonation of ionic groups an acid environment, followed by electrostatic repulsion of the residual amino groups in the PECs, causing a further

TABLE II
Maximum Solvent Uptake Capacity (*C*) in 24 h at 37°C for Membranes Prepared Using Different Chitosan Solution Flow Rates

Flow rate (mL h^{-1})	C_{water} (g g^{-1})	C_{SS} (g g^{-1})	C_{SBF} (g g^{-1})	C_{FBS} (g g^{-1})
80	35.21 ± 0.97[a]	6.24 ± 0.55[e]	4.82 ± 0.52[g]	5.4 ± 0.22[i]
120	15.95 ± 0.26[b]	5.28 ± 1.55[e]	5.63 ± 1.92[g]	5.07 ± 0.13[i]
200	21.26 ± 0.87[c]	12.51 ± 0.95[f]	7.59 ± 2.53[g]	7.87 ± 0.32[i,j]
300	39.40 ± 1.97[d]	10.78 ± 4.01[f]	7.04 ± 4.93[h]	6.24 ± 3.39[j]

Different letters indicate significant difference at 95% confidence limits (Tukey test).

TABLE III
Percentage of Mass Loss of Membranes Prepared Using Different Chitosan Solution Flow Rates During Exposure to Aqueous Solutions at 10°C for 40 Days

Flow rate (mL h^{-1})	Mass loss (%)			
	Water	SS	SBF	FBS
80	9.92 ± 0.40[a]	4.21 ± 0.94[d]	3.32 ± 0.55[e]	4.74 ± 1.26[g]
120	2.69 ± 1.02[b]	3.56 ± 1.54[d]	3.10 ± 0.30[e]	1.90 ± 0.12[h]
200	5.25 ± 1.00[c]	3.65 ± 0.18[d]	12.93 ± 1.91[f]	8.86 ± 0.58[i]
300	10.23 ± 3.50[a]	3.61 ± 0.89[d]	5.33 ± 1.86[e]	8.54 ± 2.43[i]

Different letters indicate significant difference at 95% confidence limits (Tukey test).

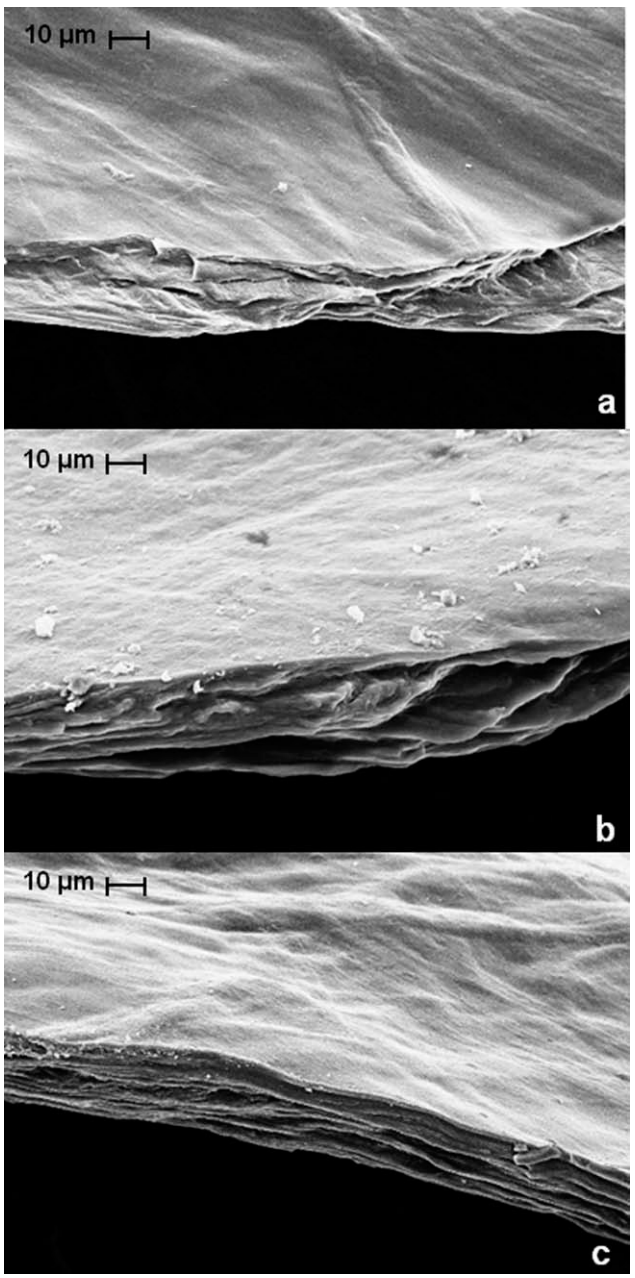

Figure 3 Cross-section of membranes prepared using different chitosan–xanthan mass ratios: (a) 0.25% Ch:0.25% X; (b) 0.375% Ch:0.25% X, and (c) 0.375% Ch:0.375% X.

expansion of the polymeric structure that made water penetration and absorption easier.

In contact with aqueous solutions, the membranes composed of chitosan and xanthan showed a discrete mass loss (Table III), probably as a result of the gradual dissolution of poorly complexed polymeric molecules, as discussed previously. The coacervates, in the form of fibrous aggregates, are not soluble in aqueous solvents, and since chitosan has low solubility in the aqueous media tested, the detected mass loss was most likely due to the dissolution of xanthan molecules not properly bond to the coacervates. Mass loss was also directly related to the fluid absorption capacity. In general, the higher mass losses were detected in the samples with higher fluid absorption; however, since the maximum loss detected was around 13%, the membranes can be considered very stable.

Because the membranes obtained at a chitosan addition flow rate of 300 mL h^{-1} had low thickness values, highest tensile strength, high fluid absorption capacity in the different aqueous media tested and adequate stability in the same media, this condition was selected for the studies involving different polysaccharide ratios. Because these membranes are

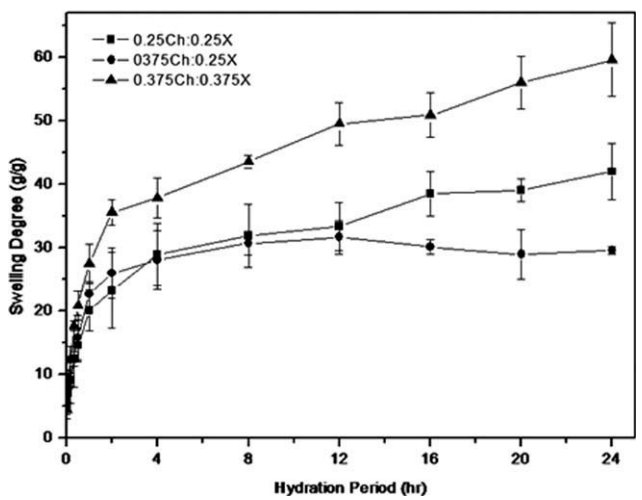

Figure 4 Hydration profile of chitosan–xanthan membranes prepared using different mass ratios of chitosan–xanthan.

TABLE IV
Thickness, Water Drainage Ability, Tensile Strength, and Elongation at Break of Membranes Prepared with Different Mass Ratios of Chitosan to Xanthan

Ch : X mass ratio (% : %)	Membrane thickness (μm)	Water drainage ability (g m^{-2} day^{-1})	Tensile strength (MPa)	Elongation at break (%)
0.375 : 0.25	159.00 ± 47.76[a]	5901.11 ± 469.41[b]	2.48 ± 0.98[d]	2.44 ± 0.97[e]
0.375 : 0.375	200.14 ± 45.23[a]	3433.62 ± 315.23[c]	2.65 ± 0.26[d]	2.22 ± 0.61[e]

Different letters indicate significant difference at 95% confidence limits (Tukey test).

potentially not cytotoxic, they could be successfully used as wound dressings or scaffolds for animal cell cultures in tissue engineering.

Influence of polymer mass ratio on membrane characteristics

Additional membranes were prepared with chitosan and xanthan solutions at 0.5 or 0.75%, resulting in suspensions with final chitosan to xanthan concentrations before drying of 0.375%: 0.125%, 0.375%: 0.25%, and 0.375%: 0.375% in addition to those initially prepared at 0.25%: 0.25%. Fragile membranes that fragmented and dissolved during the characterization procedures were obtained at the 0.375%: 0.125% ratio; hence, this condition was not evaluated further. Using chitosan 0.7% and 1% solutions and a xanthan solution below 0.5%, Argin-Soysal et al.[15] did not obtain a stable hydrogel, confirming the result reported herein. The results obtained for the membranes prepared at the chitosan to xanthan concentration ratios of 0.375%: 0.25% and 0.375%: 0.375% are shown in Figures 3 and 4 and in Tables IV–VI.

No significant differences are noted regarding the packing of the coacervates in the membranes (Figs. 1d and 3), but multiple lamellas can be observed in all preparations. Apparently, the use of different polymer concentrations did not cause major modifications in any aspect of the PEC structure obtained.

The average thickness of the membranes varied from 111.90 to 200.14 μm (Tables I and IV) proportionally to the increase in polysaccharide concentration. This behavior was expected, given that the membranes were cast using the same initial area, but different polymer masses.

It was observed that drainage ability (Tables I and IV) increased with the addition of chitosan, but when more xanthan was added, it was reduced. This happened because when more chitosan was present in the mixture, probably some parts of the xanthan chains were not involved in coacervate formation, making the structure more permeable. However, when chitosan and xanthan were added at the same ratios, the coacervates were possibly more stable and the increased membrane thickness made water permeation more difficult, decreasing the drainage rate.

A reduction in tensile strength (Tables I and IV) was noted as the polyssacharide concentration increased. This can be attributed to the fact that the increase in number of polysaccharide chains in solution (since the final mixture volume is the same) can decrease the number of ordered connections between the polymers and also the organization of crystalline zones, increasing the formation of amorphous zones.

The membranes had a high water uptake capacity (Tables II and V), varying from 24.22 to 61.23 g H_2O/g dry membrane, but lower absorption values were attained in the salt-containing solutions, as noted previously. Increasing only chitosan concentration caused a reduction in the absorption levels of all solutions, and this observation is probably associated with the fact that chitosan itself absorbs much lower amounts of aqueous solutions[21] than chitosan–alginate[19] or chitosan–xanthan membranes. In saline solution the absorption is lower than that in water due to the presence of ions in the solution that compete with it in the hydroxyl groups of the polymeric chain for the solvent. Increasing both chitosan and xanthan by the same amount, on the other hand, resulted in a massive increase in water absorption, but not of the remaining media. It was verified that in the presence of solvents of high ionic strength (NaCl solution and simulated body fluid), or high osmolality and increased concentration of proteins

TABLE V
Maximum Solvent Uptake Capacity (*C*) in 24 h at 37°C of Membranes Prepared with Different Mass Ratios of Chitosan to Xanthan

Ch : X mass ratio (% : %)	C_{water} (g g^{-1})	C_{SS} (g g^{-1})	C_{SBF} (g g^{-1})	C_{FBS} (g g^{-1})
0.375 : 0.25	24.22 ± 1.73[a]	8.68 ± 2.45[c]	3.59 ± 0.78[e]	3.50 ± 0.66[f]
0.375 : 0.375	61.23 ± 12.24[b]	13.08 ± 1.83[d]	5.20 ± 0.86[e]	4.55 ± 1.17[f]

Different letters indicate significant difference at 95% confidence limits (Tukey test).

TABLE VI
Percentage of Mass Loss of Membranes Prepared with Different Mass Ratios of Chitosan to Xanthan During Exposure to Aqueous Solutions at 10°C for 40 Days

Ch : X mass ratio (% : %)	Mass loss (%)			
	Water	SS	SBF	FBS
0.375 : 0.25	11.36 ± 0.56[a]	4.83 ± 2.27[b]	6.40 ± 1.46[c]	7.39 ± 1.15[d]
0.375 : 0.375	12.56 ± 1.74[a]	3.45 ± 0.21[b]	5.23 ± 1.15[c]	5.12 ± 0.98[d]

Different letters indicate significant difference at 95% confidence limits (Tukey test).

(the fetal bovine serum), the absorption capacity of the membranes decreased in relation to the water, probably by competition of ions and proteins for the solvent, decreasing its availability to hydrate the polysaccharide chains. Rodrigues et al.[19] verified similar capacities to absorb these solutions for chitosan–alginate membranes after 24 h. Martínez-Ruvalcaba et al.[9] reported a comparable behavior of chitosan–xanthan hydrogels in PBS buffer at pH 7.0 and saline solution for 90 min, relating it to the interaction between ions and the charges present in the polymer chains.

In the hydration profile analysis (Fig. 4) it was verified that the membranes absorbed high quantities of water for the whole period analyzed, except for the 0.375% chitosan to 0.25% xanthan preparation. Hydration of these samples, which contained more chitosan than xanthan, stabilized at around 4 h, and only discreet variations were observed during the remaining period. The samples prepared with different polymeric concentrations but the same mass ratios (0.25%: 0.25% and 0.375%: 0.375%) showed similar behaviors, indicating that the PEC was not modified under these conditions.

The samples exposed to water had mass losses of 11.36 to 12.56% (Tables VI). In comparison with the 0.25%: 0.25% formulations, no significant differences were observed. When in contact with the remaining media, except the FBS, higher membrane stability in terms of degradation was observed.

Therefore, if membranes that are thinner and more mechanically resistant in terms of tensile strength are desired, the preparations obtained with 0.25% chitosan and 0.25% xanthan are suggested. If, however, high water absorption is required, membranes obtained with 0.375% chitosan and 0.375% xanthan could be successfully employed.

CONCLUSION

The results obtained show that it is possible to produce coherent membranes by coacervation of chitosan and xanthan without the use of organic solvents or reticulating salts. Membranes with thicknesses between 112 and 200 μm, with drainage abilities between 3180 and 5900 g m^{-2} day^{-1}, tensile strengths between 2.2 and 5.2 MPa, elongations at break of up to 2.5%, water uptake capacities from 16 to 61 g H_2O/g of dry membrane and maximum mass of 13% loss in the aqueous solutions tested were obtained. According to the results, the most appropriate rate of addition of chitosan solution to xanthan solution is of 300 mL h^{-1}. The increase in chitosan to xanthan concentration ratios in the membrane from 0.25%: 0.25% to 0.375%: 0.375% provided better water absorption, but slightly reduced mechanical resistance.

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References

1. Dumitriu, S.; Chornet, E. Adv Drug Deliv Rev 1998, 31, 223.
2. Lee, J. W.; Kim, S. Y.; Kim, S. S.; Lee, Y. M.; Lee, K. H.; Kim, S. J. J Appl Polym Sci 1999, 73, 113.
3. Berger, J.; Reist, M.; Mayer, J. M.; Felt, O.; Gurny, R. Eur J Pharm Biopharm 2004, 57, 35.
4. Muzzarelli, R.; Baldassarre, V.; Ferrara, P.; Biagini, G. Biomaterials 1988, 9, 247.
5. Kumar, M. N. V. R. React Funct Polym 2000, 46, 1.
6. Niekraszewicz, A. Fibres Text East Eur 2005, 13, 16.
7. García-Ochoa, F.; Santos, V. E.; Casas, J. A.; Gómez, E. Biotechnol Adv 2000, 18, 549.
8. Kim, C.; Yoo. B. J Food Eng 2006, 75, 120.
9. Martínez-Ruvalcaba, A.; Chornet, E.; Rodrigues, D. Carbohydr Polym 2001, 67, 586.
10. Chellat, F.; Tabrizian, M.; Dumitriu, S.; Chornet, E.; Magny, P.; Rivard, C. H.; Yahia, L. J Biomed Mater Res 2000, 51, 107.
11. Chu, C. H.; Kumagai, H.; Nakamura, K. J Appl Polym Sci 1996, 60, 1041.
12. Dumitriu, S.; Chornet, S. Biotechnol Prog 1997, 13, 539.
13. Dumitriu, S.; Magny, P.; Montane, D.; Vidal, P. F.; Chornet, E. J Bioact Compat Polym 1994, 9, 184.
14. Magnin, D.; Dumitriu, S.; Chornet, E. J Bioact Compat Polym 2003, 18, 355.
15. Argin-Soysal, S.; Kofinas, P.; Lo, Y. L. Food Hydrocolloids 2009, 23, 202.
16. Phaechamud, T.; Ritthidej, G. C. AAPS Pharm Sci Tech 2008, 9, 870.
17. Popa, N.; Novac, O.; Profire, L.; Lupusoru, C. E.; Popa, M. I. J Mater Sci Mater Med 2010, 21, 1241.
18. Eftaiha, A. F.; Qinna, N.; Rashid, I. S.; Al-Remawi, M. M.; Al-Shami, M. R.; Arafat, T. A.; Badwan, A. A. Mar Drugs 2010, 8, 1716.
19. Rodrigues, A. P.; Sanchez, E. M. S.; Costa, A. C.; Moraes, A. M. J Appl Polym Sci 2008, 109, 2703.
20. Bernabé, P.; Peniche, C.; Argüelles-Monal, W. Polym Bull 2005, 55, 367.
21. Dallan, P. R. M.; Moreira, P. L.; Petinari, L.; Malmonge, S. M.; Beppu, M. M.; Genari, S. C.; Moraes, A. M. J Biomed Mater Res B Appl Biomater 2007, 80B, 394.