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Preparation of chitosan membranes for filtration and concentration of compounds under high pressure process

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Summary

Chitosan membranes of three types: i) dried in ammonia atmosphere (CSA); ii) double layer crosslinked with glutaraldehyde (CSG); and iii) prepared from aqueous-ethanolic solution and dried in the presence of ammonia vapor (CSE) were developed by casting chitosan solutions onto a glossy paper used as a support in the process of filtration under high pressures. All the membranes were characterized by infrared spectroscopy, scanning electron microscopy and by permeation experiments. Addition of ethanol to the chitosan solution decreased the time of membrane preparation and of the filtration process. The performance (solute rejection) of these membranes was found to be efficient with organic compounds such as methylene blue, truncated hemoglobin and bovine serum albumin (BSA) with molecular weights of 319.8 Da 17.7 kDa and 66.4 kDa respectively, since it retained practically 100% of the sample.

Introduction

Conventional techniques of mass separation such as distillation, crystallization, extraction with solvent etc., have been substituted by processes employing biopolymers as the essential element for the separation of molecular mixtures. These techniques must be of low cost and their use should be compatible with green technologies [1].

Chitosan, a poly-2-amino-2-deoxy-b-(1,4)-D-glucopyranose, is derived from chitin, poly-2-acetamide-2-deoxy-b-(1,4)-D-glucopyranose. Chitin is one of the most abundant natural polysaccharides, primarily obtained as a sub-product of seafood. Chitosan and its derivatives have received considerable attention due to their potential beneficial activities, such as antitumor, antiulcer, immunostimulatory, anticoagulant, antimicrobial activities and it has been applied in the biomedical and pharmaceutical areas, mainly because of its biodegradability, low toxicity, and good biocompatibility [2-9]. Chitosan has also been used as a flocculant and adsorbent in wastewater treatment and chitosan-metal complexes have been found to be much better than free

chitosan and metal complexes as antimicrobial agents against a variety of bacteria and fungi. The inhibitory effects were dependent on the property of the metal ions, the molecular weight and degree of deacetylation of chitosan and environmental pH values [10-12].

Asymmetric membranes that operate under high pressures consist of a very thin (0.1 to 1.0μm) polymer layer on a highly porous, 100 to 200μm, thick sub-layer [13]. The highly porous sublayer serves only as a support for the very thin skin and has very little or no effect on separation characteristics and the mass transfer rate of the membrane [14]. The behavior of the membrane can be evaluated in terms of applied pressure and permeation rate and, in the case of charged membranes, the separation of the ionic compound depends on intrinsic properties of the membranes as well as the electrostatic interaction between the ionic permeant molecules and the charged membrane [15-16].

Chitosan membranes can be used in separation techniques such as ultrafiltration and reverse osmosis [17-18]. The ultrafiltration process is governed by a size exclusion mechanism, solute-solute and solute-membrane interactions that are dependent on membrane surface characteristics such as hydrophilic/hydrophobic balance, electrostatic charges on both membranes, and on the nature of the solute [18-19]. Chitosan is a hydrophilic material and in the acid pH range it is positively charged due to protonation of $–NH_2$ groups [12], but in ammonia atmosphere deprotonation of the polymeric chain occurs. In ultrafiltration, the hydrophilic membranes tend to absorb less protein and suffer less flux loss due to fouling than hydrophobic membranes and, as a consequence, allow more effective protein transmission. Higher permeation fluxes and higher protein rejections have been observed when the membrane and protein charges are similar and it has been recognized that the hydrophilicity/hydrophobicity balance is not the only factor involved in membrane fouling. Indeed, protein rejection can be affected by high protein adsorption and pore narrowing as well as by electrostatic effects [15, 19-22].

This paper describes the preparation of chitosan membranes deposited on a support, focusing only on the development and preparation technique of membranes, followed by their characterization by infrared spectroscopy (IR) and scanning electron microscopy (SEM) to compare the morphologies. The performance of the membranes in the filtration of bovine serum albumin (BSA) and methylene blue was found to be satisfactory and this serves as a basis for further studies on membrane selectivity of modified chitosan membranes.

Experimental

Materials

Chitosan from the shrimp shell was purchased from Purifarma (Brazil). The deacetylation degree of chitosan was estimated as 99% by condutimetric titration and the average molecular weight was determined as 122.7 kDa by viscosity measurements. The support used was glossy paper HP 51630Z CX JetSeries CutSheet (thickness 0.39μm, 0.886 g/dm^{-2}) manufactured in the United States. The solutions used for the filtration were prepared with ultrapure deionized water. Methylene blue was purchased from Vetec (Brazil), glacial acetic acid, ethyl alcohol and glutaraldehyde from Nuclear (Brazil) and BSA A 4503 from Sigma (United States).

Preparation of Chitosan membrane dried in ammonia atmosphere (CSA)

CSA membranes were prepared by immersing the glossy paper support in 1.0 L of a solution of chitosan (2.5% (m/v)) and acetic acid (1.5% (v/v)) for 3 h. The membranes were then removed from the solution and placed in a chamber under an ammonia atmosphere at 25°C for 72 h. The resulting membranes had a thickness of 0.11mm, 0.954 g/dm^{-2}.

Preparation of chitosan membrane crosslinked with glutaraldehyde (CSG)

CSG membranes were prepared by immersing the glossy paper support in 1.0L of a solution of chitosan (2.5% (m/v)) and acetic acid (1.5% (v/v)) for 3 h. The membranes were then removed from the solution, placed on an acrylic support and dried in air. After this, the membranes were immersed in a glutaraldehyde solution (2.5% (v/v)) for 24 hours and then removed, placed on an acrylic support and dried in air for 24 h. The membranes were then immersed for a second time in the chitosan/acetic acid solution described above. The membranes were again removed, placed on an acrylic support and dried in air at 25°C for 72 h. The resulting membranes had a thickness of 0.134mm, 1.370 g/dm^{-2}. In relation to the infrared characterization, it is important to remark that after drying, the film covered an area larger than the paper and a sample of this excess film, which without the support paper was perfectly transparent, was collected for the purposes of comparison with pure chitosan using IR spectrometry.

Preparation of Chitosan membrane from aqueous-ethanolic solution (CSE)

The CSE membranes were prepared by immersing the glossy paper in 1.0 L of a solution containing chitosan (2.5% (m/v)), ethyl alcohol (50% (v/v)) and acetic acid (1.5% (v/v)) for 3 hours. The membranes were then removed and dried in a chamber under ammonia atmosphere at 25° C, for 50 hours. The resulting membranes had a thickness of 0.11mm, 0.989 g/dm^{-2}.

High pressure filtration experiments

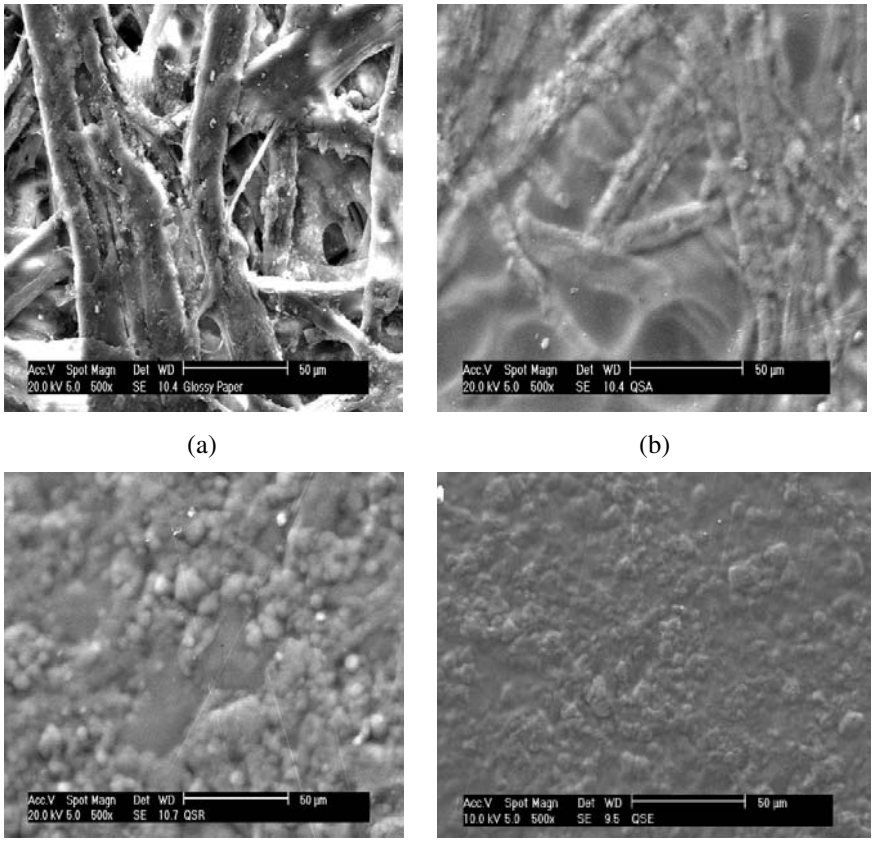
The experiments were conducted using a Sepa ST test cell equipment, which has a low retention volume. The stirred cell (7 cm^2 area and 500 rpm) supports pressures up to 1000 psi and is constructed of stainless steel and chemically resistant components. The total volume of the filtration system is 300 mL, and after filtration the remaining (retention) volume is 1 mL. Concentrations of methylene blue and of the BSA protein were determined using a UV-vis spectrophotometer. The solute rejections ($R=1-C_p/C_r$) were calculated from the absorption measurements (λ = 665 nm for methylene blue and 210-340 nm for BSA).

Results and discussion

Three types of chitosan membranes were prepared: i) dried in the presence of ammonia vapor (CSA); ii) double layer crosslinked with glutaraldehyde (CSG); and iii) prepared from aqueous-ethanolic solution dried in the presence of ammonia vapor (CSE). The membranes were stored at room temperature under normal laboratory conditions and produced reproducible results over an 18-month period.

Scanning electron microscopy of prepared membranes

Figure 1 illustrates transverse sections of the support used for the preparation of the membranes, and of the CSA, CSG and CSE membranes, respectively, with a magnification of 500-fold. The morphology of the support in the transverse section shown in Figure 1a is typical of a fibrous form. Figures 1b to 1d show the effect of chitosan addition, which clearly decreases the observed porosity. In Figure 1b, it can be seen that in the CSA membrane, the fibrous structure of the glossy paper, used as the support, has been covered with a thin film of the chitosan biopolymer. Similar SEM micrographs of the CSG and CSE membranes show that the typical fibrous form of the support has been fully covered by the chitosan films and, therefore, they are expected to be less permeable to larger particles.



(a) (b)

(c) (d)

Figure 1. SEM micrographs with magnitudes of 500 X from: (a) paper support used for membrane preparation; (b) chitosan membrane dried in ammonia atmosphere (CSA); (c) chitosan double layer crosslinked with glutaraldehyde (CSG); (d) chitosan membrane prepared from aqueous-ethanolic solution dried in the presence of ammonia vapor (CSE).

Infrared spectroscopy (IR) of chitosan membranes and crosslinked membranes

Figure 2 illustrates IR spectra in the range of 500-2000 cm^{-1} from chitosan and the crosslinked chitosan films. The bands observed in the spectrum for the chitosan film showed the presence of a characteristic band at 1655 cm^{-1}, which corresponds to the stretching of amide C=O and at 1590 cm^{-1} to the N-H deformations of a primary amine. The band at 1381 cm^{-1} is attributed to the C-H of group CH_3 of the acetamide group, which indicates that chitosan is not completely deacetylated. The band at 1078 cm^{-1} corresponds to the C-O stretching of a primary alcohol. In the spectrum for the crosslinked chitosan, the band at 1590 cm^{-1} is absent and the band at 1568 cm^{-1}, which is absent in chitosan spectrum, shows the presence of the C=N stretching, thereby confirming the Schiff base formation and crosslinking of chitosan in $-NH_2$ groups.

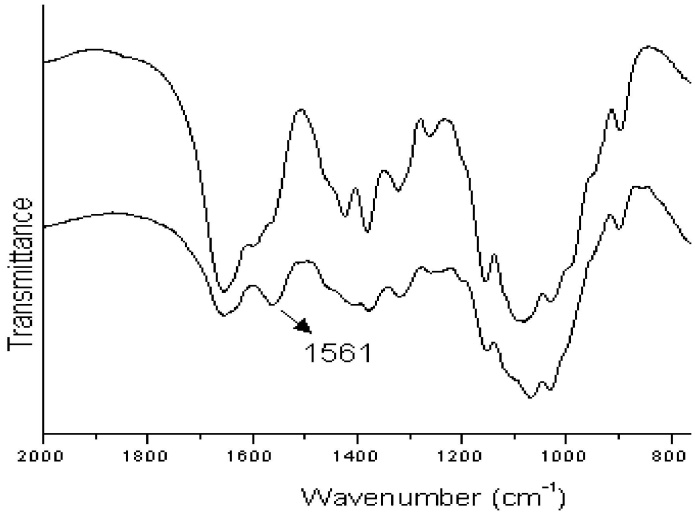


Figure 2. Infrared spectra of chitosan and crosslinked CSG film.

High pressure filtration experiments

The CSA, CSE and CSG chitosan membranes were tested in the stirred cell to verify the variation of the flux at different pressures. The flux rate of the CSG membrane increases linearly as a function of the applied pressure, and the filtration rate, at any applied pressure, follows the order CSE > CSG and CSA > CSG (Figure 3). The lowest water flux shown by the CSG membrane is consistent with the fact that this membrane has the most dense structure (Figure 1). Indeed, the results in terms of lower flux rate and higher resistance to pressure effects on the CSG crosslinked chitosan membrane is fully consistent with the improved resistance to degradation, as well as the increased mechanical force and resistance generally associated with the cross-linking process. For the CSA and CSE membranes, the flux rate does not increase linearly as a function of the applied pressure, a result indicative of membrane compaction of the spongy layer at high pressure. Clearly, the increasing effect of bulk layer compaction is greater in the CSA membrane.

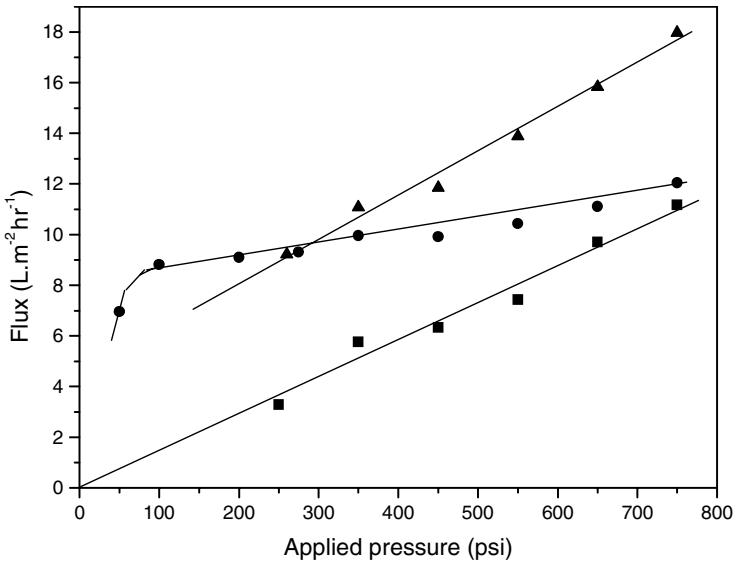


Figure 3. Variation of flux rates as a function of applied pressure for (■) CSG, (▲) CSE and (●) CSA membranes.

The reported results are reproducible to ± 9 % and consistent with the fact that the tested membranes were shown to have very low molecular weight cut off (see below), the permeate flux obtained was somewhat lower than reported for polyacrylonitrile membranes [17].

Initially, the permeability of the chitosan membranes was tested by analyzing the rejection of the organic dye methylene blue, from an aqueous solution (6.0×10^{-4} M) in the stirred cell. The measurements were replicated at least three times with a fresh membrane sample in each run and the applied pressure was 500 psi. The methylene blue rejections were calculated from the measured concentration of dye in the permeant, which allows the estimation of C_r using the mass balance equations and the initial concentration in the stirred reactor. Basically, the CSG, CSA and CSE membranes were able to fully retain the organic dye and, therefore, concentrate methylene blue in the aqueous solution.

Concentration of Proteins and Rejection by Chitosan Membranes

In order to test the chitosan membranes with high molecular weight components, an aqueous solution of BSA at a concentration of 1 g L^{-1} was tested on the stirred cell assembly to check BSA transmission through the CSG, CSA and CSE membranes, at a pressure of 500 psi. The results of the filtration of the protein solution with all membranes showed 100% retention of the BSA (66.4 kDa) sample (measurements replicated at least three times with fresh membrane samples in each run). The BSA rejections were calculated as described above for the organic dye, by measuring the concentration of protein in the permeate (absorbance measurement).

Similarly, using a low molecular weight truncated hemoglobin from *Herbaspirillum seropedicae* (17.7 kDa), analysis of the filtrate and of the concentrate were indicative of 100% retention with all three chitosan membranes. It is important to remark that the

analysis of the proteins in the filtrate and in the concentrate was performed via SDS-PAGE under reducing conditions in 16% acrylamide gels and the proteins were visualized using $AgNO_3$. Even with this highly sensitive detection method, we were not able to detect any protein in the filtrate. All three chitosan membranes showed identical behavior with 100% retention, and analysis of the concentrate against molecular weight markers of 45 kDa, 31 kDa, 21.5 kDa and 14.4 kDa was consistent with the 17.7 kDa molecular weight of the truncated hemoglobin.

Clearly, CSA, CSG and CSE membranes are appropriate for the concentration of organic compounds in aqueous solutions. The effective concentration of samples of methylene blue, truncated hemoglobin and bovine serum albumin (BSA) with molecular weights of 319.8 Da 17.7 kDa and 66.4 kDa respectively, indicates that the chitosan membranes can be used as general purpose membranes for the concentration of organic samples.

Conclusion

CSA, CSG and CSE membranes were found to be efficient in the process of retaining organic compounds such as methylene blue and proteins such as truncated hemoglobin and BSA (molecular weights of 319.8 Da, 17.7 kDa and 66.4 kDa, respectively) and in all cases retained practically 100% of the sample. It is important to remark that the chitosan membranes can be easily prepared, with low cost, and were found to be satisfactory for the concentration of organic compounds under high pressure. The results are indicative that the chitosan membranes can be used as general purpose membranes for the concentration of organic samples and can serve as a prototype for further developments on membrane selectivity of modified chitosan membranes.

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References

1. Merkel TC, Freeman BD, Spontak RJ, He Z, Pinnau I, Meakin P, Hill AJ (2002) Science 296: 519
2. Huang R, Du Y, Yang J (2003) Carbohydr Polym 51:431
3. Qin C, Zhou B., Zeng L., Zhang Z, Liu Y, Du Y, Xiao L (2004) Food Chem 84: 107
4. Kobayashi M, Natanabe T, Suzuki S, Suzuki M (1990) Microbiol Immuno 34: 413
5. Liu H, Du Y, Wang X, Hu Y, Kennedy JF (2004) Carbohydr Polym 56: 243
6. Jeon YJ, Kim SK (2000) Cabohydr Polym 41: 133
7. Roller S, Covill N (1999) Inter J Food Microbiol 47:67
8. Gonçalves VL, Laranjeira MCM, Fávere VT, Pedrosa RC (2005) Polímeros 15: 6
9. Nascimento A, Josué A, Laranjeira MCM, Fávere VT (2001) J Microencapsulation 18: 679
10. Geremias R, Pedrosa RC, Benassi JC, Fávere VT, Stolberg J, Menezes CTB, Laranjeira MCM (2003) Environ Technol 24: 1
11. Fávere VT, Laus R, Laranjeira MCM, Martins AO, Pedrosa RC (2004) Environ Technol 25: 861

12. Wang X, Du Yumin, Fan L, Liu H, Hu Y (2005) Polym Bull 55: 105
13. Carvalho R.B. de, Borges C.P., Nobrega R (2001) Polímeros 11: 65
14. Bungay PM, Lonsdale HK, Pinho MN, (1986) Synthetic Membranes: Science, Engineering and Applications, D. Reidel Publishing Company, Dordrecht
15. Gómez-Espinoza H, Lin SW (2001) Polym Bull 47: 297
16. Hanza A, Chowdhury G, Matsuura T., Sourirajan S. (1995) J Appl Polym Sci 58:613
17. Musale DA, Kulkarni SS (1996) J Memb Sci 111: 49
18. Yang T, Zall RR (1984) J Food Sci 49: 91
19. Vourch M, Balannec B, Chaufer B, Dorange G (2005) Desalination 172: 245
20. Amy G, Cho J (1999) Wat. Sci.Technol. 40: 13
21. Cho JW, Amy G, Pellegrino J (2000) Desalination 127: 283.
22. Rivas BL, Pereira ED, Mondaca, MA (2003) Polym Bull 50: 327