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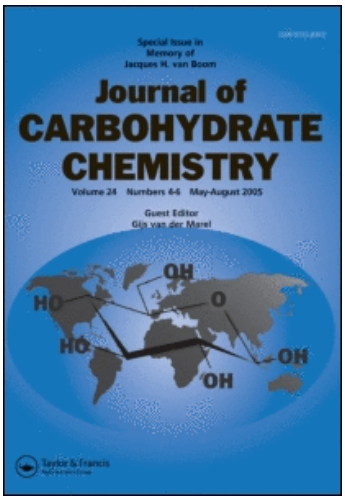

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Thermal Characterization of Chitosan-Grafted Membranes to be Used as Wound Dressings

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Thermal Characterization of Chitosan-Grafted Membranes to be Used as Wound Dressings

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Six different membranes previously prepared by the authors by graft copolymerization of acrylic acid (AA) and 2-hydroxyethylmethacrylate (HEMA) onto chitosan were characterized.

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The graft copolymerization of the vinyl monomers onto chitosan was confirmed by FTIR and DMTA. The chitosan used was also characterized by DMTA, which allowed addressing some questions related to the typical thermal transitions of this biopolymer. The swelling capacity of the membranes was also determined. The chitosan-graft-AA-graft-HEMA with an equimolar ratio of HEMA and AA kept good swelling capacity without compromising its physical stability, which suggests it to be the best matrix for drug delivery systems.

Keywords Graft copolymerization, Chitosan, Wound healing

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INTRODUCTION

Until the early 1960s, the general practice in wound care was to keep the wound as dry as possible. Wound treatment was then revolutionized when it was found that a wound would heal faster when a "moist" dressing was applied rather than the traditional "dry" dressings. Natural skin is recognized as the ideal wound dressing and so the development of moist dressings was based on the interest of developing substitutes of skin with 85% water content and inherent permeability. Polysaccharides, like chitosan and its derivatives, have been considered to be advantageous in their application as a wound dressing material since they may, by themselves, actively participate in the process of wound healing.[1] Hyaluronic acid and chitosan can be used for this purpose. A forerunner of present-day polysaccharide dressings was used by the ancient Egyptians when they applied disaccharides in the form of honey to wounds sustained in battle.[2]

Chitin, the second most abundant natural polysaccharide, extracted primarily from shellfish sources, is a linear homopolymer composed of β(1-4)-linked *N*-acetyl glucosamine.[3] Partial deacetylation of chitin results in the production of chitosan, which is a polysaccharide composed by copolymers of glucosamine and *N*-acetyl glucosamine[4] (Fig. 1).

Chitosan possesses several characteristics favorable for promoting rapid dermal regeneration and accelerated wound healing suitable to be applied as a wound dressing material.[5] One hypothesis to explain the ability of chitosan to enhance wound healing is related to its biodegradability.[6] This property, associated with its biocompatibility, nontoxicity, adsorption properties, film-forming ability, bioadhesivity, and antimicrobial activity against fungi, bacteria, and viruses, in addition to its hemostatic effect make chitosan an excellent biomaterial to treat wounds and scars.[7–15]

Recently, there has been a growing interest in performing chemical modification of chitosan to improve several of its characteristics and, therefore, widen its applications. Among the several available methods, the graft copolymerization is one of the most attractive, since it allows the formation of functional derivatives by covalent binding of a molecule, the graft, onto the chitosan backbone. Another important feature of this method is that the

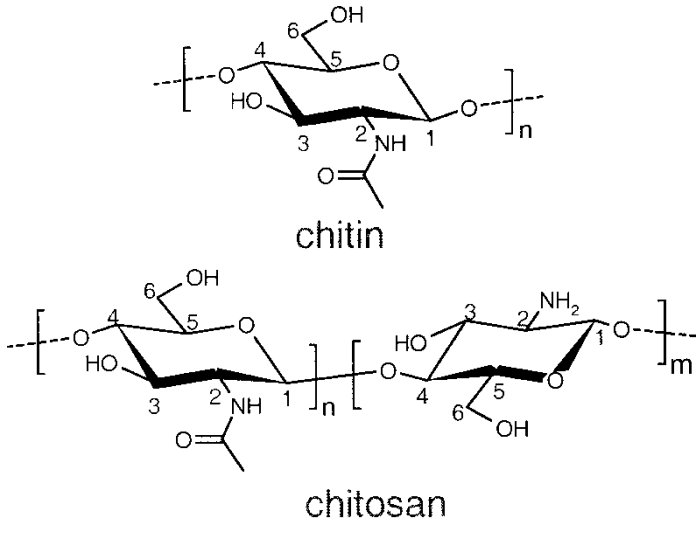

Figure 1: Chitin and chitosan partial structures.

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mucoadhesivity, biocompatibility, and biodegradability of the chitosan are maintained, although it becomes possible to obtain molecules of grafted chitosan presenting different properties from the original molecule.[16] Chitosan bears two types of reactive groups that can be modified by grafting: the C-2 free amino groups on deacetylated units and the hydroxyl groups in the C-3 and C-6, either in acetylated or deacetylated units[6,17] (Fig. 1).

The poly(AA), which is biocompatible and with antibacterial properties, is widely used in adhesives and superabsorbent materials due to its pendant carboxylic groups.[18–20] Polymers grafted by AA become highly hydrophilic materials and interesting matrixes for drug delivery systems. They make a good wound dressing due to their content in water ("moist" dressing) and, since membranes can absorb large contents in water, they are able to retain higher concentrations of drug into their matrix when dried membranes are immersed on drug aqueous solution. Some of previous studies have shown that the presence of HEMA in copolymers improves the biocompatibility of these materials.[21,22]

The main objective of this study was to characterize chitosan-based membranes for wound treatment with dual effects: to accelerate wound healing due to the bioactivities of chitosan itself, and simultaneously the polymeric matrix should present the possibility to act as a matrix to delivery drugs to prevent or treat bacterial infections. For this purpose, new materials synthesized by the authors by grafting vinyl monomers, AA and HEMA, onto chitosan[23] were characterized by thermal analysis, and comparisons between them and chitosan itself were established. This work showed that the membranes grafted both with AA and HEMA presented better characteristics than

chitosan-AA and chitosan-HEMA since their swelling capacity and physical stability seem to be the most suitable for the pretended application.

RESULTS AND DISCUSSION

The graft copolymerization was a random and heterogeneous process as judged by FTIR (Fig. 2). Side chains could be covalently linked to the C-2 free amino groups in deacetylated units or to the hydroxyl groups in the C-3 and C-6 carbons either of acetylated or deacetylated units.[6,17] They are formed by the arrangement of the HEMA and AA monomers either in blocks or randomly. Despite these variables, the success of graft copolymerization reactions was confirmed by FTIR and DMTA.

Infrared Analysis

Figure 2 represents the FTIR aligned spectra of all the samples.

The absorption at $1720\,cm^{-1}$ was attributed to the C═O asymmetric stretching of the ester of HEMA monomer, confirming that M2, M4, M5, and M6 samples were grafted with polyHEMA. The very intense characteristic band at $1560\,cm^{-1}$ is due to the C═O asymmetric stretching of the carboxylate anion from AA grafted onto M3, M4, M5, and M6 chitosan membranes. This was reconfirmed by another sharp peak at $1405\,cm^{-1}$, which is related to the symmetric stretching mode of the carboxylate anion.[24,25] The salt form of the free carboxylic group is expected since the membranes were neutralized with sodium hydroxide solution to remove them from the plates.

Swelling Capacity Evaluation

It is expected that the biological and physiological potential of chitosan application on wound healing would increase with the easy water swelling of chitosan. Graft copolymerization of chitosan appears as a method to control this characteristic of the synthesized materials.

The results obtained in swelling studies are shown in Figure 3.

M3 sample, the membrane with the highest content in AA, showed the highest swelling capacity ($SR = 2860\%$), which was about 14-fold more water absorbent than that of the control (M1), but it was fragile to handle.

This elevated water retention capacity can be explained by the ionization that chitosan and AA can suffer together in their side groups. When chitosan was dissolved in the acrylic acid solution, some of the amino groups became positively charged due to the reaction with the proton dissociated from the acrylic acid, $NH_2 + H^+ \rightarrow NH_3^+$. Simultaneously, the grafted PAA became negatively charged: $COOH \rightarrow COO^- + H^+$. Consequently, repulsive forces between charges along the chains of both chitosan and grafted PAA chains

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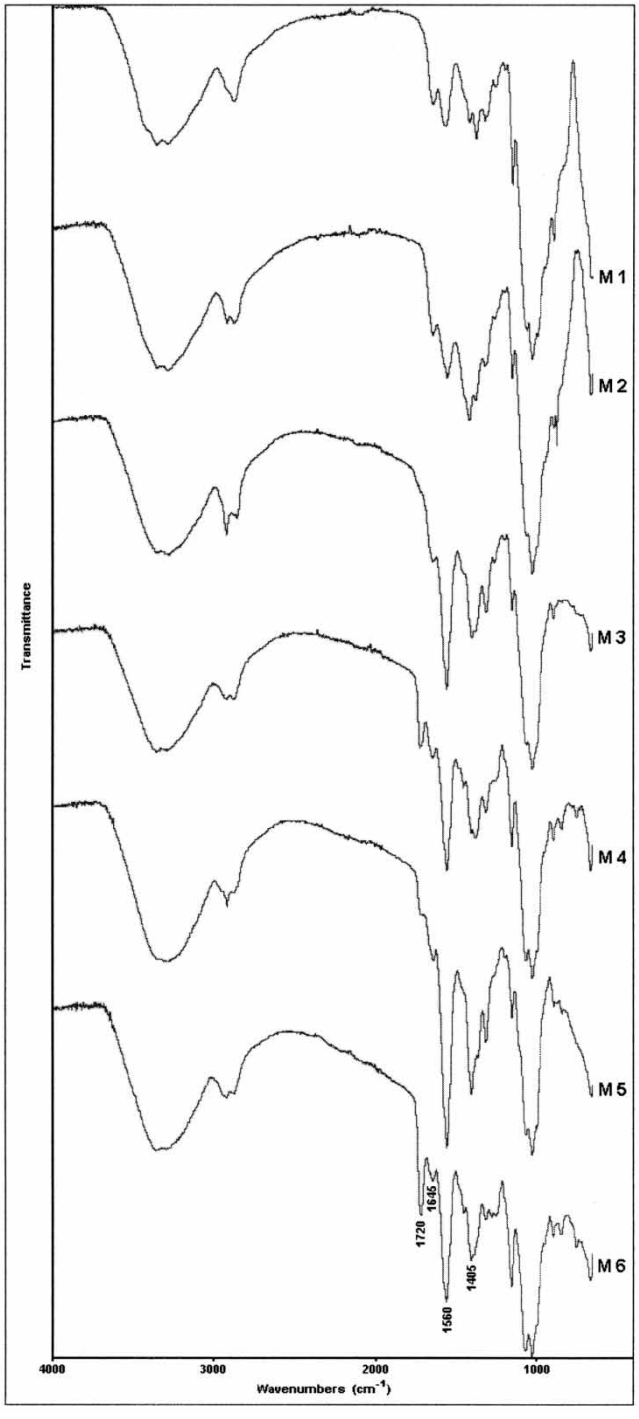

Figure 2: FTIR aligned spectra of **M1**, **M2**, **M3**, **M4**, **M5**, and **M6** samples.

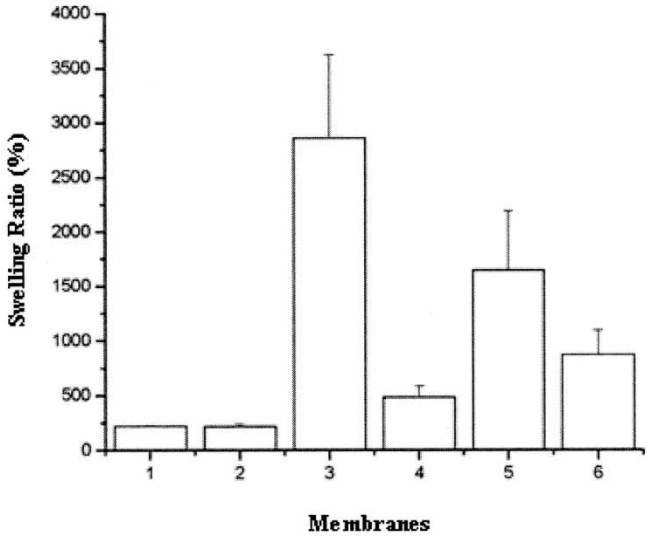

Figure 3: Swelling capacity of the membranes.

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are established, resulting in a very extended and rigid structure. However, when the extended PAA chains intersect with the chitosan chains, ionic force between the negative-charged carboxylate groups and the positive-charged amino groups hold the three-dimensional structure together and work as cross-linking points. Considering that both of them are hydrophilic polymers, they can absorb large amounts of water and keep these water molecules in this pseudo cross-linked structure.[26]

The M2, the membrane with the highest HEMA content, presented the lowest swelling degree ($SR = 220\%$), which was almost the same as that of the control ($SR = 223\%$). Although HEMA was added to chitosan in M2 in the same molar ratio that AA was used in M1, the swelling results were significantly different. In fact, HEMA does not present the ability to ionize like AA, and for that reason the interactions that occur between chitosan and grafted PAA cannot take place between chitosan and HEMA.

Sample M4 did not present a significant increase in swelling capacity when compared with control, and M5, despite its high swelling degree, did not show improved physical properties when compared to M3, remaining a very fragile membrane. However, the main purpose of mixing HEMA and AA on graft reactions was to obtain membranes with good swelling capacities as well as suitable physical structure. In this context, chitosan membrane grafted with equimolar ratio of HEMA and AA (M6) had swelling properties balanced according to the composition of each monomer. It was possible to

absorb fivefold more water than with a chitosan membrane (M1) without impairing its physical stability. The large standard deviations measured in some triplicates reflected the heterogeneity of the graft reactions. Depending on the membrane portion taken for the test, the results could differ drastically between themselves due to the random insertion of the vinyl side chain grafted onto chitosan.

Thermal Properties

Chitosan

It has been assumed that the dynamic mechanical thermal analysis (DMTA) has enough sensibility to detect all the relaxation temperatures. In this technique the storage modulus (elastic response, E′) and loss modulus (viscous response, E″) of the sample, under oscillating load, are monitored against time, temperature, or frequency of oscillation. Their ratio (E″/E′) defines the loss tangent (tan δ).

The thermal changes are extremely important since they can help to understand the physical and physiochemical behavior of chitosan with expected shifting in the relaxation temperatures. For this reason, the DMTA appears as an extremely important tool for the characterization of chitosan. This assumption is related to the great sensibility in the detection of small changes in the polymer structure. The determination of the chitosan thermal properties was difficult to carry out, mainly due to the problems associated with the sample preparation and its hygroscopicity. These biopolymers have the ability to absorb water, creating hydrogen bonds,[27] which affect strongly the polysaccharide thermal and mechanical properties, resulting in a substantial depression of the glass transition temperature.[28]

A considerable number of papers dealing with the influence of water on the relaxation properties have been published over the last few years.[29–32] Such characteristic justifies clearly the fact that so different values of T_g have been reported with differences that sometimes can be as large as 50°C. The water content in chitosan also depends on the degree of deacetylation (DD), becoming higher for lower values of DD.[27]

The thermomechanical behavior of chitosan used in this work was examinated by DMTA, using as reference a sample containing 15% of moisture.

Figure 4 shows that the sample has five important changes corresponding to five peaks of tan δ: at 0°C, 72°C, 131°C, 201°C, and finally 292°C.

The first peak at 0°C is related to the chain movements induced by the water presence.[33] The peak at 72°C can be attributed to some water-induced relaxation due to the referred chitosan hygroscopicity. The second transition at 131°C[27,34] is normally ascribed to the glass transition temperature. Concerning the peak at 200°C, it is difficult to recognize as consequence

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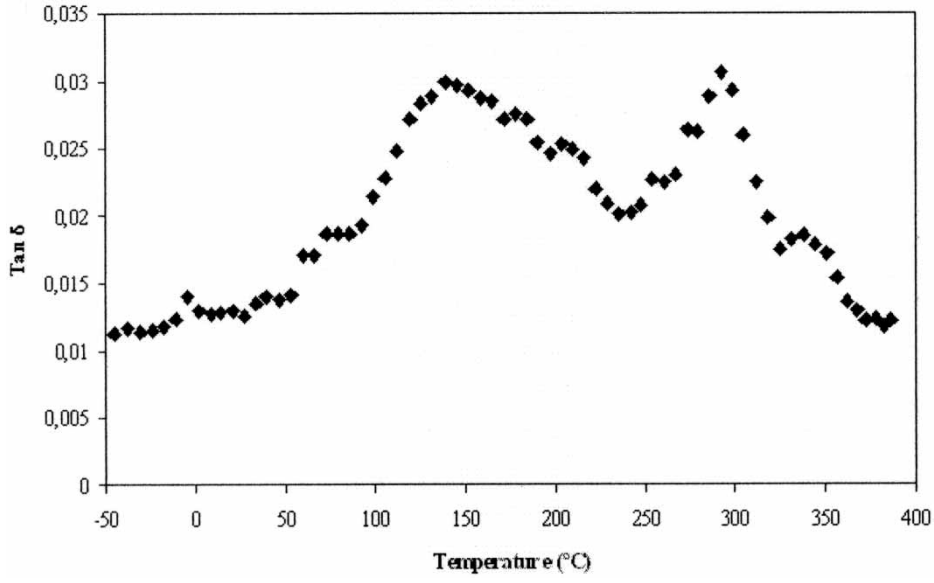

Figure 4: Tan δ for a pure chitosan sample, single cantilever bending, heating rate 10°C, frequency 1 Hz.

of this any molecular movement. Some authors have reported that the T_g of chitosan is around 200°C,[35] which is very close to the degradation temperature onset. One possible explanation for the peak at 200°C is some liquid-liquid transition, which corresponds to the motion of very long segments or even the whole chain.[36] The final peak at 300°C is amply recognized as related to the decomposition temperature.[37,38] It starts generally around 230°C, and can also be confirmed by using the thermogravimetric data published[39] or using our own TGA data (see below). The start temperature for decomposition can also be reported (with slight different values) to the dependence of the acetyl content and the degree of polymerization that influences the thermal stability. A small peak at 350°C was also detected, and most probably is due to the main chain scission.

It should be noted that frequently the transition temperature around 15°C is reported in the literature as the β transition of chitosan.[34] Normally, this transition was ascribed to the rotation of the hydrated methyl groups or local motions of chain segments in the disordered region. However, it was impossible to detect the refereed peak in our samples. The absence of this peak has also been reported after the blending of chitosan with cellulose, due to interaction of side groups ($-CH_2OH$) on chitosan with $-OH$ on cellulose, which subsequently displaces the hydrogen bonding water.[34]

Figure 5 represents the results of DSC and TG/DTG using the same chitosan studied by DMTA.

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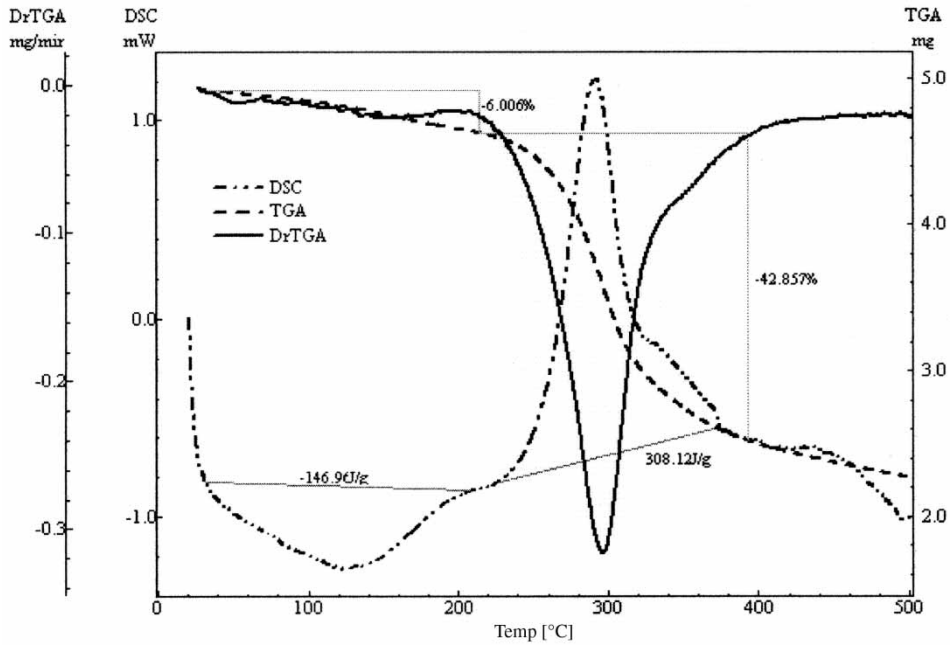

Figure 5: Results of DSC and TG/DTG for chitosan obtained under nitrogen atmosphere and heating rate of 10°C.

The first mass loss occurred ($\Delta m = 6.00\%$) between 27°C and 214°C and is due to sample moisture. The decomposition started around 214°C, representing the loss of 42.86% of the total mass. Concerning the DSC data, it shows the appearance of endothermic event between 30 and 210°C ($\Delta H = 147\,J \cdot g^{-1}$, Tpeak = 122°C), which can be related to the water linked to the chitosan. Again, another transition (exothermic) appears between 230°C and 375°C, which naturally represents the polymer degradation ($\Delta H = 308.1\,J \cdot g^{-1}$, Tpeak = 292°C) as observed by DMTA. The results obtained by DSC and TGA are additional proof about the accuracy and precision obtained in the

Table 1: Molar ratios of the membranes (M1–M6) components.

Sample	Chitosan (mmol)	HEMA (mmol)	AA (mmol)	Molar ratio HEMA:AA
M 1	1,86	—	—	—
M 2	1,86	12	0	—
M 3	1,86	0	12	—
M 4	1,86	9	3	3 : 1
M 5	1,86	3	9	1 : 3
M 6	1,86	6	6	1 : 1

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DMTA analysis. The important match obtained between the temperatures obtained by the three different techniques should be stressed, the temperature of decomposition being an outstanding example.

In order to evaluate the potential influence of the water content in the chitosan samples, a study was carried out on the dependence of the T_g with the water content. In amorphous polymers, this transition represents motions of long-chain segments and represents the freedom for movement of the long chains in the biopolymer.

Figure 6 depicts the dependence of the thermal properties with the moisture content for four samples conditioned in atmospheres with different moisture levels.

The water present in the chitosan can be split in two different types: the free water known as "freezing" water, which can express the water bulk properties in the thermograms; and the "nonfreezing" water, which is difficult to remove under mild temperatures even when vacuum is applied.[26] From the molecular interaction point of view, the difference between these two "types" of water is that in the case of the free water there is no interaction with the polymer, which explains the reason why it is so easy to remove, whereas the bound water establishes hydrogen bounds with the polymer. The described difference can be noted when even after drying the chitosan, the peaks associated with the "nonfreezing" water do not disappear. Considering the curve correspondent to the sample with 150% of moisture, the first peak is located around 0°C, being attributed to the rotation of the hydrated methyol groups or local motions of chain segments in disordered regions. The second

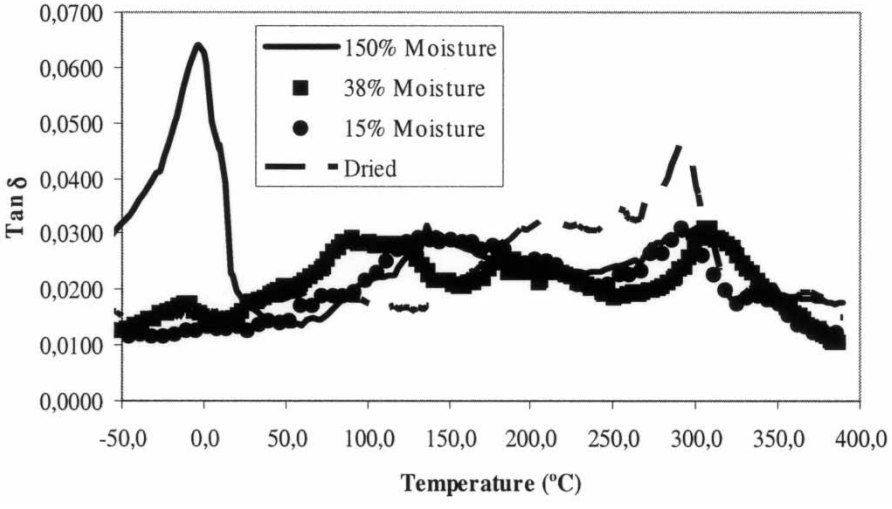

Figure 6: Tan δ for a pure chitosan sample for different levels of moisture, single cantilever bending, heating rate 10°C, frequency 1 Hz.

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one, related to small movements induced by the water presence, appears around 75°C, followed by the peak around 100°C, associated with the free water present in the chitosan. The peaks related with the transitions temperatures above 100°C remain in the sample place when compared with those described for Figure 6. This could be due to the fact that the water evaporated during the experiment.

When analyzing the dried sample, it was possible to confirm that after drying the film, the peaks related to the water presence disappeared, except the peak at 75°C which, as referred before, is related with "nonfreezing water." Moreover, the glass transition temperature is close to 200°C, which shows unequivocally the influence of the water in the thermal behavior of this biopolymer. Another important observation is the fact that the α transition region has shifted to lower temperatures with the increasing of water content. The water plays the role of a common plasticizer, increasing markedly the chain movement.

Regarding the onset of degradation, as it is expected, the temperature did not change with the water content due to the fact that all the water presented is released before reaching the degradation.

To further elucidate the exact relaxation region that corresponds to an α transition temperature, the DMTA spectra were investigated at different frequencies.

Figure 7 shows the shifting of the $\tan \delta$ to higher temperatures with the increasing frequencies as expected for any thermal-activated relaxation process.

The special sensibility of the glass transition temperature to different frequencies allowed us to identify, without any doubt, the T_g peak. It was possible to confirm again that the depicted peak at 131°C in Figure 1 corresponded effectively to the glass transition temperature. This possibility represents an important characteristic of the DMTA, considering the impossibility to confuse this transition with structural changes as crystallization or melting point.

Graft Copolymers

The DMTA represents a powerful technique to evaluate the capacity of miscibility of different compounds. This can be an important help in characterizing the graft copolymers based on chitosan. It is normal to assume that the detection of a single peak in tan δ is a sufficient criterion to assume the miscibility, when the compounds do not have close T_g values.

To avoid possible errors induced by the presence of water and considering the results shown related to the moisture influence in the chitosan thermal properties, all graft copolymers prepared were dried before the DMTA tests under the same conditions.

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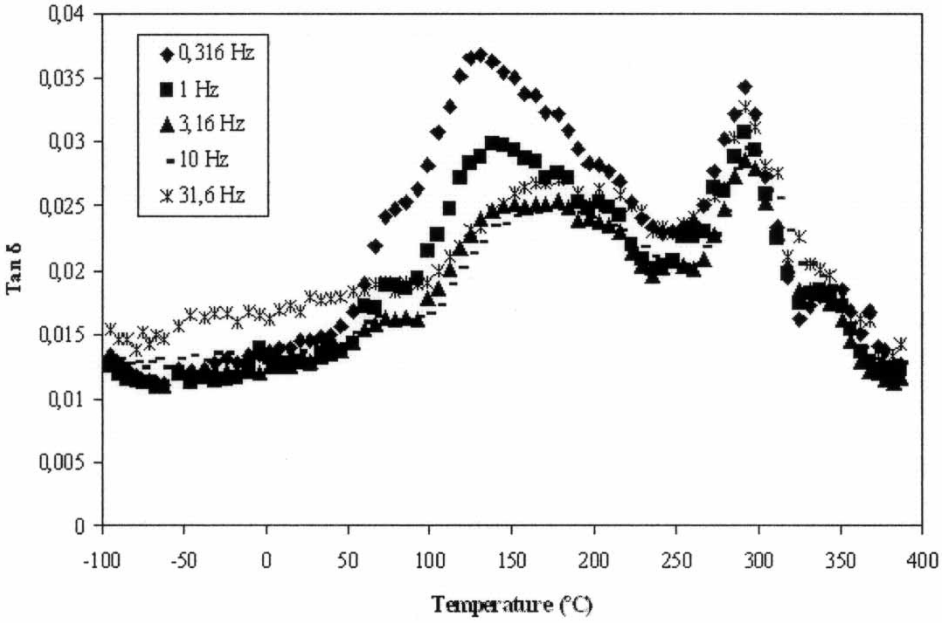

Figure 7: Multifrequency tan δ for chitosan film.

PHEMA

The thermogram depicted in Figure 8 shows the presence of a single and sharp peak in the α-transition region for the chitosan-g-PHEMA copolymer. This observation allowed us to consider a perfect miscibility between these two materials.

Figure 8 suggests that the peak around 75°C disappeared, indicating a different water behavior in chitosan when PHEMA is present. Another important characteristic of the copolymer prepared is the lower intensity of the peaks at 0°C and 72°C that, as explained before, are related with water induction movements. This shows a decrease in the capacity to retain water due to the introduction of the PHEMA, following the trend observed in the swelling tests.

PAA

The PAA is widely used in adhesives and superabsorbents. This is due to its pendant carboxylic groups. The possibility of producing a copolymer of chitosan and AA can increase the range of potential applications of such materials as superabsorbent, as biocompatible, and with antibacterial properties.[19]

Figure 9 shows a higher T_g value for the graft copolymer with AA when compared with chitosan. This observation is related, most probably, with the reaction between amino groups in chitosan and carboxylic groups in PAA, resulting in the formation of a stiffer material.

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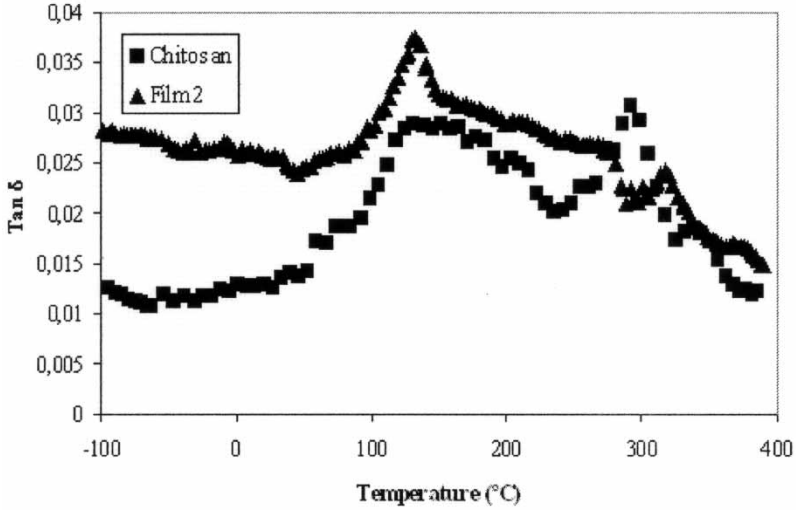

Figure 8: Tan δ curves of chitosan and chitosan-g-PHEMA.

PAA/PHEMA—Several Molar Ratios

The results related to M4 are indicated in Figure 10.

The spectrum showed a clear phase separation in the graft copolymer prepared, which may indicate that when large amounts of PHEMA were used, which were not eliminated by Soxhlet extraction, the system could not keep the homogeneity. When the HEMA concentration was higher than the one of AA, two distinct phases were formed in the copolymer. It could be noticed also that the onset of degradation started at just around 300°C. The graft-copolymer heterogeneity was also observed by the broadened glass transition region, ascribed to the presence of chitosan-rich regions and PHEMA- and PAA-rich regions. Concerning the membrane M5, the degradation curve showed the presence of two different peaks, which may indicate the existence of ungrafted chitosan that started to degrade at lower temperatures. Finally, for membrane M6, the α relaxation of the graft copolymer chitosan-g-PHEMA-g-PAA was shifted to higher temperature compared with PAA and PHEMA themselves, and to lower temperature compared with chitosan itself, which indicated some interaction between all components. However, a small peak exactly for 100°C was also detected. This behavior was related to the water presence, and it was interesting compared to the results obtained in the swelling tests. Those have shown that between M4, M5, and M6, the latter one presented the higher swelling degree.

From these results, it is also possible to suggest that an excess of HEMA in the presence of AA during the graft copolymer results in the formation of two different phases. In an opposite trend, when excess of AA was used in the presence of PHEMA, just one phase was detected.

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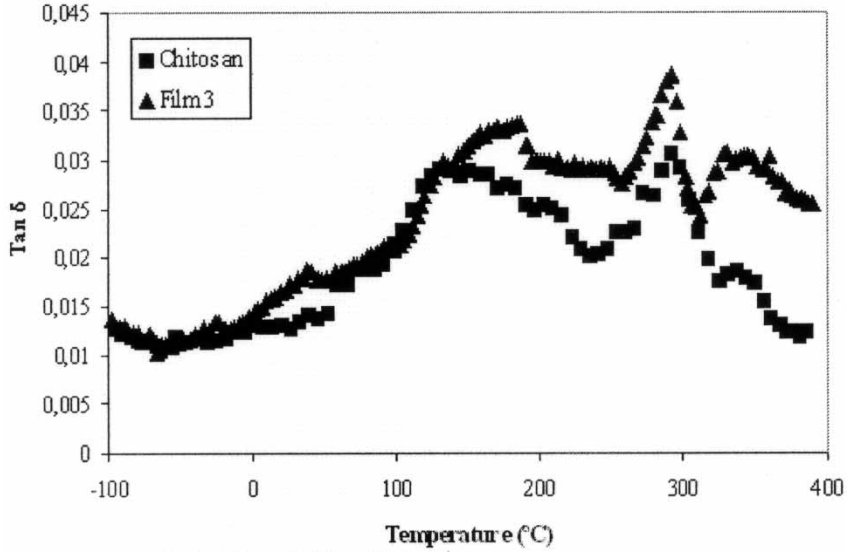

Figure 9: Tan δ curves of chitosan and chitosan-g-PAA.

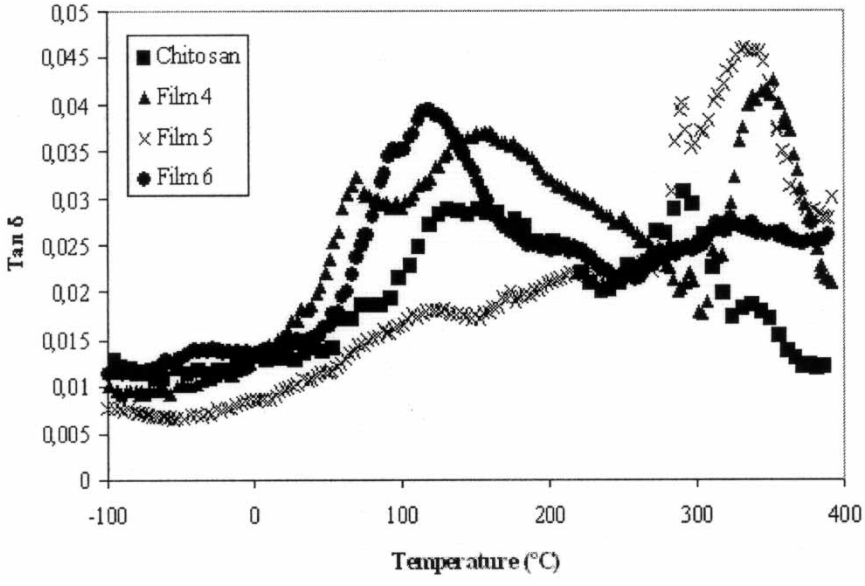

Figure 10: Tan δ curves of chitosan and chitosan graft copolymers with different relative amounts of PHEMA and PAA.

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The difference between the values of the degradation temperature onset for the different copolymers prepared and the chitosan seems to suggest a possible cross-linking process between the two monomers studied and the chitosan.

EXPERIMENTAL

A 2% (w/v) chitosan stock solution was prepared in 1% (v/v) acetic acid aqueous solution. Fifteen millilitres of chitosan stock solution was stirred with 50 mg of cerium ammonium nitrate for 10 min. AA and HEMA monomers, which were used in different molar rates according to Table 1, were added to this solution and stirred until complete homogenization. The resulting solutions were transferred to Petri dishes and allowed to react for 24 h at 55°C. Films were further obtained and removed from the Petri dishes by adding 1% sodium hydroxide aqueous solution. They were extracted in a Soxhlet for a 6-h reflux using distilled water as a solvent to remove the homopolymer or nonreacted monomers. Finally, the membranes were dried at rt on Teflon® surfaces.[23]

The M1 sample corresponded to the ungrafted chitosan membrane, which acted as the control. M2 and M3 samples represented chitosan membranes grafted by single monomers of HEMA and AA, respectively. Vinyl monomers were also used together on graft reactions at different molar ratios to generate M4, M5, and M6.

Infrared Analysis

FTIR analysis of the membranes was performed on a Magma–IR™ Spectrometer 750 from Nicolet Instrument Corp. equipped with Golden Gate Single Reflection Diamond ATR. The spectra were recorded on an average of 128 scans at a resolution of 4 cm^{-1}.

Swelling Capacity Evaluation

To determine the swelling ratio (SR), the membranes were primarily dried until constant weight (W_d). These samples were then immersed in distilled water at rt for 48 h. The swollen samples were then removed from the solution, quickly wiped with filter paper, and weighed (W_s). The swelling ratio was evaluated by application of equation (1).

$$Swelling\,ratio(\%) = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \quad (1)$$

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Thermal Properties

Thick specimens ($15.20 \times 7.45 \times 1.10$ mm) were analyzed by Dynamical Mechanical Thermal Analysis (DMTA). A Triton Tritec 2000 analyzer was used in the Constrain Layer Damping mode, with a standard heating rate of $10^{\circ}C\ min^{-1}$, at frequency of 1 Hz and a displacement of 0.05 mm. The glass transition temperature (T_g) was determined as the peak in tan δ (tan $\delta = E''/E'$) where E'' and E' are the loss and storage modulus, respectively, derived from DMTA. All samples were dried for 24 h at 55°C before thermal analysis.

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CONCLUSIONS

Swelling properties are important to mimic the skin's moisture and to incorporate drugs into the polymeric matrix. However, too much water impairs the physical stability of the membranes. The chitosan membranes containing only HEMA (M2) tend to be thicker and have more wrinkles than those composed exclusively of chitosan (M1). Chitosan membranes grafted exclusively with AA (M3) are able to absorb large contents of water, and drastically increase their volume, which makes this film very fragile for handling. Thus, it was expected that chitosan membranes grafted with both monomers are able to mix their properties to obtain membranes with ideal swelling and mechanical and physical profiles. In fact, all the membranes containing AA were able to swell, but M6, which was grafted by equimolar ratio of HEMA and AA, kept good swelling capacity without compromising its physical stability. These conclusions were also taken from the DMTA traces, which allowed confirming the results described above. Moreover, and as expected, these tests proved to be extremely useful in the characterization of chitosan. Concerning the graft copolymers with HEMA and AA, the DMTA showed the possibility to prepare this new material with no phase separations. Finally, the concordance obtained between the different thermal analyses carried out should be stressed.

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