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Development of Biodegradable Films Based on Chitosan/Glycerol Blends Suitable for Biomedical Applications

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Abstract

In the present work, chitosan/glycerol blends were used to prepare biodegradable films with potential application in dermal treatment. While the presence of glycerol gives the hydrating effect, the chitosan characteristics contribute to dermal regeneration. Swelling degree measurements, water vapor permeability determinations, mechanical tests, scanning electronic microscopy and Fourier transform infrared spectroscopy analysis; biodegradability studies, thermogravimetric and differential scanning calorimetry analysis were used to analyze the influence of the incorporation of significant amounts of glycerol in film properties. The obtained results suggested that the glycerol addition provide the films with the required properties for the intended application related to dermal treatment. Increasing the glycerol concentration, a cluster effect produced by the glycerol molecules surrounding the macromolecular chains led to a decrease in the swelling degree and an increase in the WVP along with improvements in mechanical properties.

Keywords: Chitosan; Glycerol; Biodegradable films

Introduction

Chitosan is a biopolymer produced by deacetylation of chitin which is mainly obtained from crustacean shells [1]. It is a polycationic polymer soluble in diluted acidic solutions due to the protonation of the amino groups. The biocompatibility, biodegradability and non-toxicity characteristics of this polysaccharide allow its use in pharmaceutical, cosmetics and food industries. The manufacture of edible films or food packaging materials based on chitosan has been widely studied at laboratory level [2,3].

The use of chitosan for dermal applications in the treatment of skin injuries has been recently explored. It is an excellent candidate for this application because it has antimicrobial and anti-inflammatory properties [4,5].

Chitosan provides a non-protein matrix with a 3D tissue growth capability for tissue engineering. Applied as a film or gel, it promotes dermal regeneration producing a faster healing. The biological mechanism involved in this process has been already explained [6-8].

Chitosan dissolved in acid solutions presents filmogenic properties, allowing the preparation of films by a casting-solvent evaporation process.

Lactic acid has been used as the solvent for dissolving chitosan in powder as it also improves film flexibility [9]. This organic acid is accurate to prepare bio-adhesive films useful for dermal applications because it is biological safe, is a natural constituent of the human body and is proven to be non-irritant to the skin of rabbits [10]. A biomaterial suitable to cover the skin injuries must have specific characteristics regarding the permeability of gases, the capacity of absorbing the exudate, adequate mechanical properties and the possibility of loading with pharmaceutical drugs.

Permeability to gases, such as water vapor, is a very important characteristic when a film is evaluated for dermal applications. Lactic acid has been used in the preparation of films in order to obtain high water vapor permeability [11].

In this research, we have selected glycerol because of its high boiling point and the high hydrogen bond ability that leads to a good interaction with chitosan macromolecules assuring its retention in the polymer matrix [12]. Plasticized chitosan films with glycerol are frequently used for the production of hydrocolloid based edible films [13,14]. This polyol, which is a byproduct in biodiesel production, has an adequate plasticization efficiency and stability compared to other common plasticizers such as poly (ethylene glycol) or propylene glycol [15]. In particular, glycerol is adequate for biomedical applications because of its highly efficient hydrating and moisturizing effect on skin [16].

The polycationic properties of chitosan provide the possibility of interacting with other anionic polymers or with the hydroxyl groups existing in the glycerol molecules.

There are several publications analyzing the properties of chitosan edible films after the addition of glycerol but, to the authors knowledge at the time of writing, no work has been published using significant amounts of glycerol for wound injury treatment as it is proposed in this investigation [3,12,13,15].

In this work, the physicochemical properties of films containing a high content of glycerol with potential application in dermal treatment were investigated. Fourier Transformed Infrared Spectroscopy (FTIR) was used to analyze the interactions obtained from the glycerol addition. Differential Scanning Calorimetry (DSC) and Thermogravimetric Analysis (TGA) were applied to study the thermal stability and the thermal transitions occurred due to glycerol incorporation. Scanning Electron Microscopy (SEM) analysis, swelling tests, mechanical experiments and water vapor permeability measurements were used to

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evaluate the use of films with a significant amount of glycerol for skin injury treatment.

Materials and Methods

Materials

Chitosan of medium molecular weight (Batch SLBF6034V Brookfield viscosity 1%, 1% acetic acid: 505 cps) was provided by Aldrich Chemistry (USA). A deacetylation grade of 83%, (proportion of primary amino groups in the polymer backbone) was determined by conductimetric titration of the chitosan dissolved in clorhidric acid solution with standardized sodium hydroxide aqueous solution.

Glycerol (87% purity) and lactic acid (purity >85%) were purchased from Cicarelli (Argentina). All chemicals used in this work were of analytical grade. Deionized water was obtained by means of a Millipore Simplicity System.

Films preparation

A 3% w/w chitosan solution was prepared by dissolving chitosan (CH) powder in an aqueous solution of lactic acid (1% v/v) with magnetic stirring.

The filmogenic solution was prepared by mixing the CH solution and the glycerol (G) with mechanical stirring. G:CH mass ratios were selected ranging from 0 to 200%. In order to degas the polymeric material, the resulting mixture was centrifuged at 4000 rpm for 10 min at room temperature.

Chitosan films were obtained by means of a casting-solvent evaporation method using flat polypropylene Petri dishes of 55 mm of diameter. A constant mass/surface ratio of 231.5 mg/cm^{-2} was used. Petri dishes were placed at 50°C for 1 h in an oven and then kept under atmospheric conditions for 1 week in a chamber until constant weight.

Films characterization

Films thickness determination: Films thickness was determined using a digital hand micrometer Schwyz (Argentina, ± 0.001 mm). Each film was measured in five different positions including the center. An average value was reported and used in permeability and mechanical properties determinations. All the films were conditioned at 25°C for 48 h, inside a desiccator containing a saturated saline solution of sodium bromide which provided a relative humidity of 57.7%.

Scanning electronic microscopy (SEM) analysis: Films were conditioned in desiccators containing silica gel and subsequently mounted on metal grids and finally covered with a thin layer of gold. Edge surfaces of the films were examined with a Karl Zeiss Supra 40 SEM (Germany) with a field emission gun operated at 3 kV. The micrographs were taken at magnifications between 70 and 50000X.

Mechanical tests: Uniaxial tensile tests were carried out in an INSTRON dynamometer Model 5982 (USA) equipped with a 1 kN load cell following ASTM D 882–02 standard recommendations. Before testing, films were conditioned at 25°C for 48 h, inside a desiccator containing a saturated saline solution of sodium bromide (relative humidity of 57.7%). Crosshead speed was set at 10 mm/min. Nominal stress-strain curves were obtained and from these curves, tensile parameters values were determined: tensile strength (σ_{max}) and strain at break (ε_b). All mechanical tests were carried out at room temperature by quintuplicate. Average values and their deviations were reported.

Fourier transform infrared spectroscopy (FTIR): A Shimadzu IR Affinity-1 (Japan) spectrometer was used to obtain the infrared spectra of films at room temperature using the attenuated total reflectance (ATR) model in the range of 400-4000 cm^{-1} using 45 scans at a resolution of 4 cm^{-1}.

Swelling tests: In order to determine the swelling from water uptake, dry films were immersed into deionized water at 25°C and weighed at fixed times. The experimental mass swelling degree (Q) was calculated using equation (1):

$$Q = \frac{w_{wetfilm} - w_{dryfilm}}{w_{dryfilm}} * 100 \tag{1}$$

where, $w_{wet\ \mathrm{film}}$ and $w_{dry\ \mathrm{film}}$ are the weights of the water-swollen sample and the dry sample, respectively.

Q was calculated as grams of water per gram of sample. Tests were performed by duplicate.

Water vapor permeability (WVP): Water vapor permeability was obtained gravimetrically in accordance with the desiccant method described in ASTM E 96:00. Films were sealed on top of an acrylic permeation cell with an internal diameter of 50 mm and a depth of 35 mm containing calcium chloride. The cells were placed in a chamber with a constant humidity of 75% and a temperature of 25 ± 1°C.

Cells were weighted once a day during one week. The slope of the water uptake versus time was obtained using a non-linear fitting. Water vapor permeability (WVP) was calculated with equation (2):

$$WVP = \frac{mX}{\Delta t A S (\mathrm{R}_e - \mathrm{R})} \tag{2}$$

Where m is the weight of water absorbed by the permeability cell, X is the average film thickness, Δt is the elapsed time for weight change, A is the transfer area, S is the saturation vapor pressure of water at the test temperature (25°C) and R_e and R are the relative humidity at the test chamber (75%) and inside the permeation cell (0%) expressed as a fraction, respectively.

Tests were made in duplicate. Average values and their deviations were reported.

Thermal analysis:

Thermogravimetric analysis (TGA): Thermal degradation processes were investigated using a Shimadzu TGA-50 analyzer (Japan). Measurements were carried out by heating the sample from 25°C to 500°C in inert nitrogen atmosphere at a flow rate of 30 mL/min, with a heating rate of 10°C min^{-1} using a sample weight of 5 ± 0.1 mg. Differential thermogravimetric analysis (dTG) was also recorded.

Differential scanning calorimetry (DSC): DSC thermal profile was carried out using a Shimadzu DSC-TA-60-Plus Instrument (Japan). Samples (5 ± 0.1 mg) were sealed in hermetic aluminum pans and heated at a constant rate of 10°C/min. The DSC tracings were performed from 20°C to 350°C. During data collection, inert atmosphere was maintained by injecting nitrogen at a flow rate of 30 mL/min.

Biodegradability in soil: Biodegradation was tested in soil under controlled laboratory conditions (25°C, 25% humidity and pH=7). Samples were placed onto the soil located in plastic containers which were covered by aluminum foil in order to keep the films free of dirt.

Biodegradation was controlled taking photographs and during the first weeks, FTIR tests were also performed to analyze which chemical groups were first involved in the degradation process.

Results and Discussion

Films thickness

It was found that all the films, regardless the glycerol content, were homogenous and translucent.

After storing the films at atmospheric temperature, no mass loss was recorded as a consequence of glycerol evaporation. This is in agreement with the experimental results reported by Quijada-Garrido et al. [12] and Suyatma et al. [15] regarding the mass loss by evaporation of films prepared with different glycerol:chitosan mass ratios.

It was observed that the films average thickness increased with the glycerol content varying from 100 to 250 ± 1 µm (Table 1) in agreement with the results of others [14,17].

Chitosan is a linear hydrophilic macromolecule that produces films structured in layers. Due to the glycerol hydrodynamic radius [12], the G molecules tend to increase the spacing between the chitosan macromolecules located in each layer instead of separating them. Plasticizer molecules surrounding the polymer molecules interact by hydrogen bonds at specific sites (-OH, -NH_2). As G content increases, clusters of plasticizer molecules are generated surrounding the first polyol molecules attached to the polymer chains producing a network of G molecules linked by hydrogen bridges [12]. This would explain the obtained trend of thickness values with glycerol content.

Another factor that influences film thickness is the water absorbed by the matrix. Taking into account the hydrophilic character of G, its increase favors the absorption of ambient moisture with the consequent increment of thickness [13]. TGA results also confirmed the above hypothesis. The mass loss recorded up to 100°C was 14.1%, 15.6%, 17.6% and 19.8% for films prepared with G:CH mass ratios of 0, 50%, 100% and 200%, respectively (Table 1).

SEM analysis

Scanning electronic microscopy was used to analyze the surface morphology of the films. All surfaces were smooth and homogeneous as it has been already reported for CH films. The presence of the plasticizer reduces the number of structural disruptions such as cracks and pores [18]. In order to evaluate the effect of glycerol addition on films morphology, edge surface micrographs were also obtained. The denser structure observed with the highest G content compared to neat CH film could be appreciated in Figure 1. These observations were in agreement with the already mentioned clustering effect.

Mechanical properties

Figure 2 shows stress-strain curves obtained in uniaxial tension tests. For neat chitosan (sample G:CH (0:1)), ductile behavior was observed presenting a yield point followed by strain softening and a plateau before final fracture. Samples with glycerol, on the other hand, exhibited a typical elastomeric behavior characterized by non-linear stress-strain curves and high elongation at break values (more than 2000% increase for the maximum glycerol content). This is an important result relevant to the intended application of the films in dermal treatment as a high flexibility film is capable to copy the skin surface morphology and warrant a good contact. Tensile parameters values are listed in Table 2 along with their deviations (Table 2).

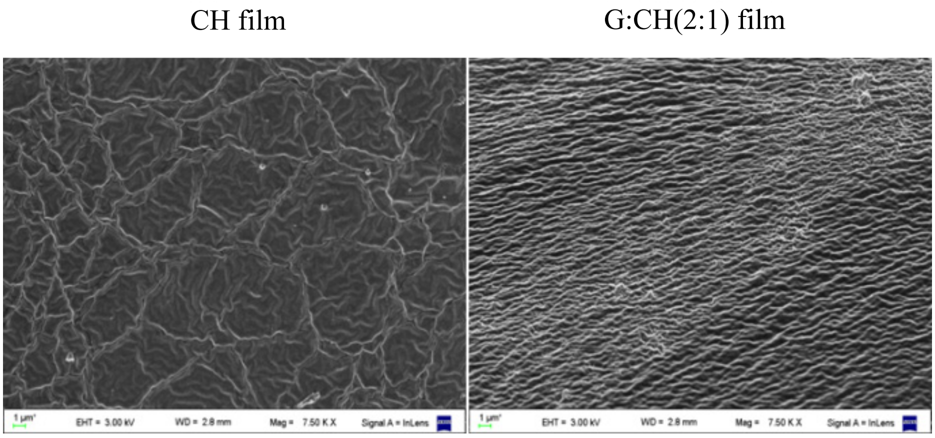
An increase in the concentration of glycerol yielded to a significant decrease in tensile strength in agreement with previous reports using glycerol and polysaccharides [19] (Figure 2).

Furthermore, a good linear relationship (correlation coefficient (R^2)=0.9989) between strain at break values and glycerol/chitosan mass ratio values was observed (Figure 3).

Changes in mechanical properties from the addition of glycerol are the result of the weakening of the intermolecular forces between adjacent chitosan chains, increasing the free volume and decreasing the mechanical strength. The small size of glycerol molecules allows them to be readily inserted between the chitosan chains [20]. Hence, the increased concentration of glycerol led to a reduction of σ_{max} due to lower intermolecular interactions by allowing more sliding chains and increasing the elongation of the films [21].

Film (G:CH)	X (mm)	WVP (g/ Pa h m)
(0:1)	140 ± 11	$(0.68 \pm 0.02).10^{-6}$
(0.5:1)	136 ± 16	$(1.21 \pm 0.02).10^{-6}$
(1:1)	181 ± 8	$(2.17 \pm 0.42).10^{-6}$
(2:1)	250 ± 16	$(4.28 \pm 0.25).10^{-6}$

Table 1: Thickness values (X) and water vapor permeability values (WVP) for the different films investigated.



CH film

G:CH(2:1) film

Figure 1: SEM micrographs of edge surfaces of films prepared without glycerol (left image) and with 200% of glycerol (right image).

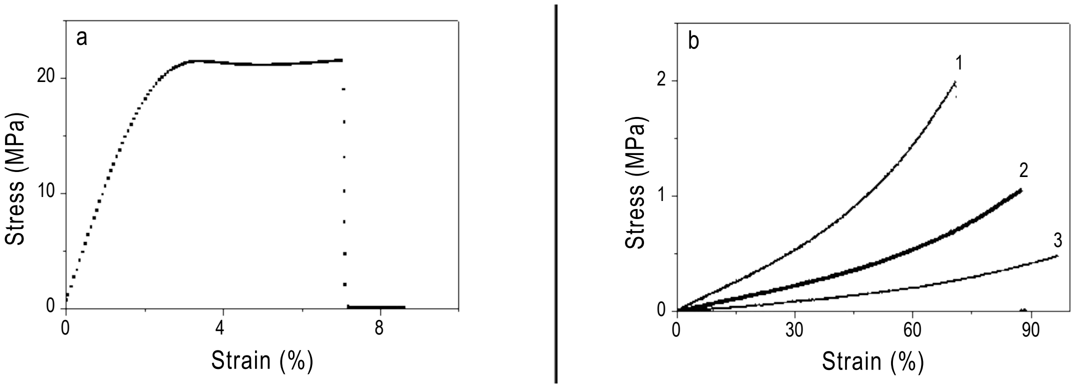


Figure 2: Stress-strain curves obtained in uniaxial tensile tests. a) G:CH (0:1); b–1) G:CH (2:1), b-2) G:CH (1:1), b-3) G:CH (0.5:1).

Film (G/CH)	σ_{max} (MPa)	ε_b (%)
(0:1)	21.1 ± 2.0	4.5 ± 1.6
(0.5:1)	1.8 ± 0.5	71.5 ± 5.3
(1:1)	0.9 ± 0.3	78.1 ± 10.3
(2:1)	0.5 ± 0.1	93.1 ± 8.2

Table 2: Tensile parameters values for the different films investigated.

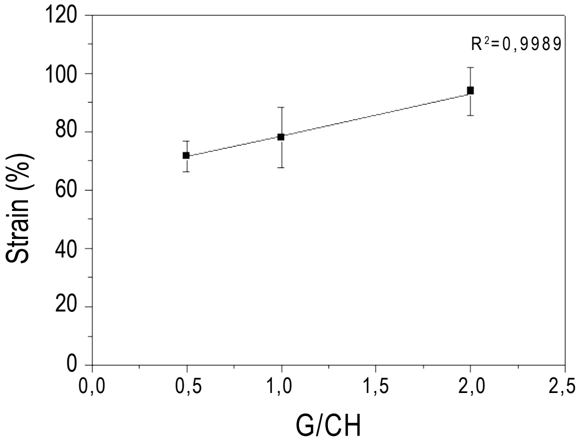


Figure 3: Linear relationship between strain at break values and G:CH mass ratio values.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

The effect of the glycerol addition was also studied by FTIR analysis. The films infrared spectra are shown in Figure 4.

The spectrum of neat CH presented three characteristic peaks between 925 and 1025 cm^{-1} that were attributed to saccharide structure [22].

As a consequence of using glycerol in the preparation of the films, some interactions were generated between CH and G modifying the recorded FTIR spectra. In the mentioned wave number range, it was possible to observe two shifts from the G addition. The first one was the shift of the peak located at 936 cm^{-1} to 925 cm^{-1} after the glycerol addition related to the symmetric stretching vibrations of the alcoxyl group [23].

The second one was the shift of the peak corresponding to the symmetric stretching vibrations of the alcoxyl group (C-O-C) at 999 cm^{-1} to 993 cm^{-1} using the highest G concentration [3]. For CH films, the carbonyl (C=O-NHR), amine (NH_2) and protonated amine (NH_3^+) bands are situated in the region between 1400 cm^{-1} and 1700 cm^{-1} [24]. The FTIR spectrum of CH films exhibited a peak at 1535.5 cm^{-1} corresponding to the amide II and a band centered at 1581 cm^{-1} related to the amine group. When the films were prepared with G, the amide II peak corresponding to NH bending vibration overlapped with the band belonging to the amine group [25]. The peak corresponding to the amide II from the addition of G shifted from 1535.5 cm^{-1}(G/CH: 0) to 1568.42 cm^{-1} (G/CH: 0.5), 1574.14 cm^{-1} (G/CH: 1) and 1580.56 cm^{-1} (G/CH: 2). The shift to higher wavenumbers indicates more hydrogen bonding interactions between chitosan and glycerol generated as a consequence of more available –OH groups [26].

The peak located at 1727 cm^{-1} suggested the presence of the carbonyl bands belonging to lactic acid (existing in the range of 1718-1729 cm^{-1}) [3,27].

The peak located at 2847.65 cm^{-1} was due to the C-H stretching. It is used as an indicator of the interaction of glycerol with chitosan,

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starch or more glycerol molecules [26]. Liu et al. [26] observed a shift for the mentioned peak towards lower wavenumbers when the glycerol concentration increased due to a slight weakening of glycerol–glycerol interactions. For the significantly higher G content used in this work, in contrast, we found a shift to higher wavenumbers: the peak was located at 2876.44 cm^{-1} and for the highest concentration at 2887.86 cm^{-1}. This could be related with the formation of glycerol clusters surrounding the chitosan molecules [12]. A broad band between 3000 and 3600 cm^{-1} was observed in CH films as a consequence of OH-O stretching vibration that overlaps the N-H stretching vibration [26].

It was found that this broad band was more intense for higher glycerol concentrations. As a consequence, the resulting area of the band grew with the G content indicating a higher intensity of hydrogen bonds which could be related with more OH groups provided by the G molecules [3] (Figure 4).

Swelling tests

Polysaccharides usually have a strong affinity for water because of the presence of significant amount of hydrophilic groups. As films, they can be easily hydrated. In order to evaluate a potential dermal application, swelling experiments are necessary to analyze the film stability during the swelling process because of the exudate produced during the healing process.

The hydration properties of polysaccharides depend on primary and supra macromolecular structure which are influenced by the presence of glycerol molecules. As shown in Figure 5, the swelling degree of chitosan films was affected by the glycerol content. The film prepared without G could not reach an equilibrium swelling degree and after some time in contact with water, the film broke apart.

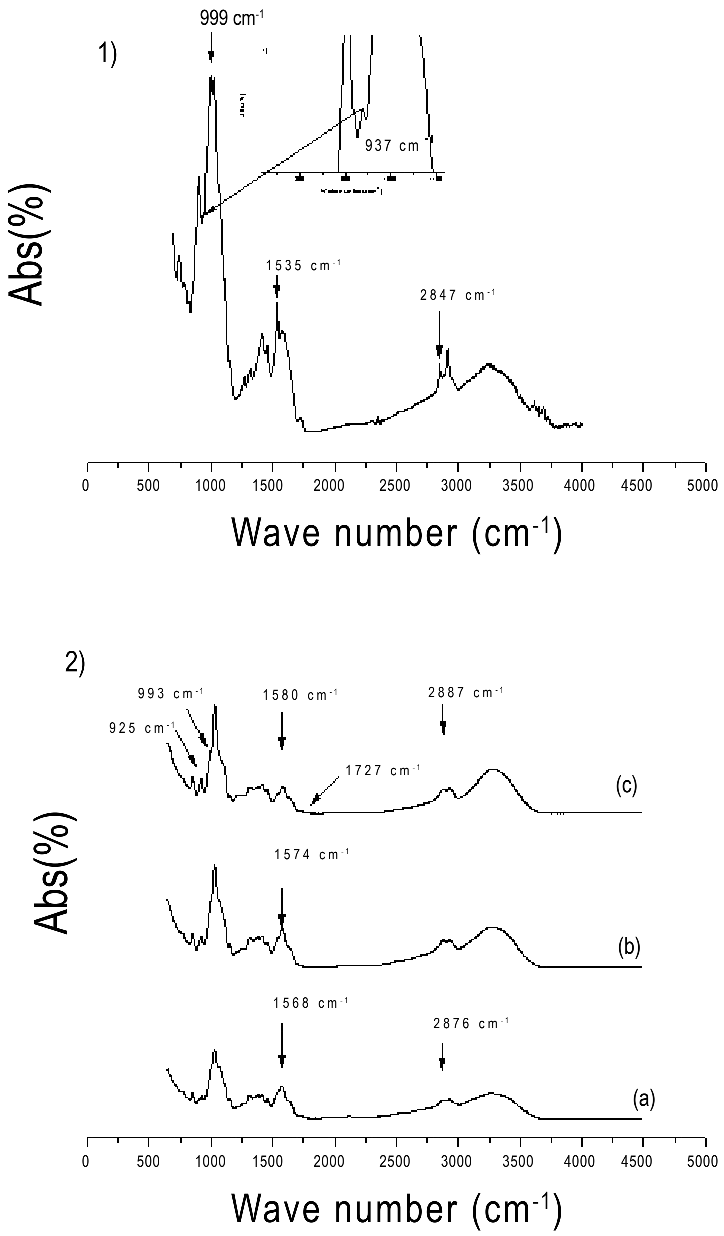

Figure 4: FTIR spectra for the different films investigated. a) G:CH (0:1), b) G:CH: b-1) (0.5:1), b-2) (1:1), b-3) (2:1).

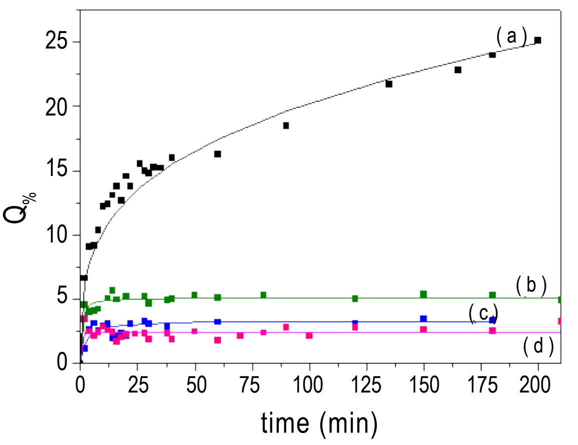

Figure 5: Swelling degree values for the different films as a function of exposure time. (a) G:CH (0:1) (b) G:CH (2:1), (c) G: CH (1:1), (d) G:CH (0.5:1).

Increasing the amount of glycerol, the obtained films displayed a higher equilibrium swelling degree. The amount of absorbed water at each time in films prepared with G was always lesser than the values registered with neat chitosan films in agreement with the results presented by Lavorgna et al. [28]. The hydrophilic character of glycerol justifies the increasing swelling degree as its content grows, which has been previously reported [3]. The introduction of increasing amounts of glycerol favored the dimensional stability of the films. This behavior could be associated to the establishment of the cross-linked network induced by hydrogen intermolecular bonds between CH and G and between G molecules. In the presence of high G concentrations, there are hydrogen bonds between the G molecules involving the G already linked to CH macromolecules, giving place to a clustering effect [12].

The presence of glycerol led to the formation of more hydrogen bonds in the matrix film, as it was confirmed by FTIR analysis (Figure 4). These interactions were responsible for the limited swelling behavior compared to the films prepared without G (Figure 5).

Water vapor permeability (WVP)

In order to use our films in dermal applications, a high WVP is required. This reflects the easiness of water vapor to pass through the film [29]. The glycerol content significantly affected the water barrier properties of the obtained films. There are contradictory reports regarding the influence of G addition in CH films depending on the G concentration and the molecular weight and deacetilation degree of the polymer. In this work, as the G concentration increased, the film WVP became higher (Table 1) in agreement with several reports [3,30].

The observed WVP increase from the incorporation of G was a consequence of an increase in the free volume between polymer chains due to decreasing attractive intermacromolecular forces, producing a polymeric matrix more permeable [31,32].

Thermal analysis

Thermogravimetric analysis (TGA): The thermal decomposition temperature of the films was measured by thermogravimetric analysis. TGA loss weight curves of chitosan and chitosan:glycerol films and their derivative curves are shown in Figures 6a and 6b, respectively.

In the temperature range explored, two steps were observed in films free of G and 3 in films prepared with G.

For both kinds of films, with and without G, the first thermal event produced a loss weight up to 120ºC as a consequence of the removal of initial water absorbed [33]. The registered weight loss of this first step depended on the G content. For films without G, the value reached up to 14.1% while for films prepared with G, the weight loss varied between 15.6% (with 50% G), 17.6% (100% G) or 19.8% (200% G). Experimental results confirmed the influence of the hydrophilic glycerol molecule on the absorption of water molecules in the films, due to the tendency to form hydrogen bonds between both molecules, as it was already discussed in the analysis of film thickness [3].

First peak changes related to the area and/or position are linked to physical and molecular variations produced by interactions between the materials used to obtain the film [33]. It was found that the peak area increased and shifted towards higher temperatures with G content. Due to the cluster effect already mentioned, water molecules interacted with the OH groups provided by the G molecules diminishing the interaction with the CH macromolecule. Taking into account that the interaction between water molecule and the OH group was stronger than the interaction between water and the amine group, the shift to higher temperature was justified.

The second weight loss was registered only for films with G. From the dTG plot (Figure 6b), it was possible to notice that the decomposition temperature shifted from 200ºC to 183ºC as the G content decreased.

The last event corresponds to a thermo oxidative process that may be related to the decomposition of amine groups belonging to chitosan macromolecules [33,34].

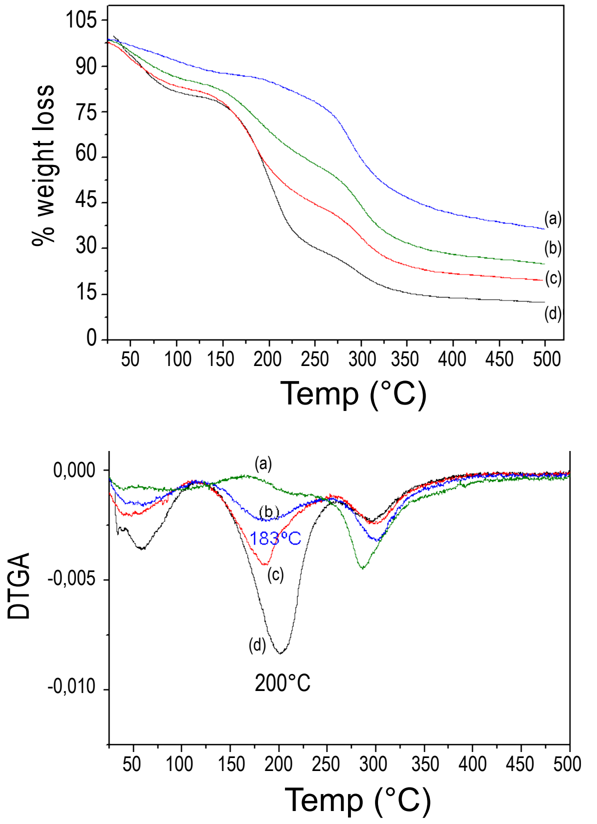

Figure 6: a) Thermogravimetric (TGA) curves for films with different content of glycerol; (1) G:CH (0:1), (2) G:CH (0.5:1), (3) G:CH (1:1), (4) G:CH (2:1). b) Differential thermogravimetric (DTG) curves for G:CH samples under a nitrogen atmosphere: (1) G:CH (0:1) (2) G:CH (0.5:1) (3) G:CH (1:1), (4) G:CH (2:1).

It was registered near 300°C due to the chemical degradation and deacetylation of chitosan [28].

The addition of G led to films more thermally stable since the maximum degradation temperature shifted from 287°C in films without G towards 300°C when they were prepared with G. This temperature was related to the degree of cross-linking (Figure 6).

Differential scanning calorimetry (DSC) analysis: Figure 7 shows the first run DSC curves obtained for the films prepared without G and with 200% of G. Differences in peak area and location were recorded depending on the composition of the sample. The first endothermic peak was related to the water presence. It was found a shift from 79.87°C (0% G) to 112.44°C with 200% G which correlated with TG results. The hydrogen bonds between the water molecules existing in the films and the CH or G hydroxyl groups are stronger than the hydrogen bonds between water and the amino groups of the CH macromolecules. Thus, a higher temperature was necessary to remove such water molecules [33] when the G content became higher (Figure 7).

Biodegradability tests

One advantage of films prepared with biodegradable materials is related to the reduction of environmental pollution. The physical changes in surface morphology of films were qualitatively studied by means of optical microscopy and FTIR. Figure 8 presents a film of neat CH and Figure 9 shows the changes produced on the surfaces of films after 4 and 18 weeks in contact with fertile soil. A progressive change on the surface of samples including cracks, holes, color changes and microorganisms appearance was visually observed. Microorganism development gradually spread and covered the surface of the samples. Because of the microorganism attack, it was impossible to follow the degradation gravimetrically.

Films without G were completely degraded after 18 weeks in soil but visual changes were only appreciated after the second week. Microorganism proliferation was enhanced by the humidity of films which was favored by the G content.

During the degradation process, produced under the influence of soil humidity and microorganisms, FTIR spectra showed some structural changes.

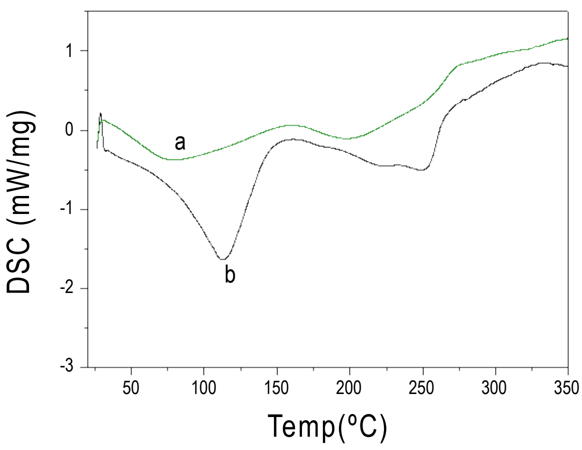

Figure 7: Differential scanning calorimetry (DSC) thermograms of chitosan:glycerol films: (a) G:CH (0:1), (b) G:CH (2:1).

Figure 8: Image of a chitosan film prepared without glycerol.

Figure 10 shows the variations in the FTIR spectrum after 2 weeks for films prepared with the maximum amount of G. The same trend was observed for all the samples (not shown).

The degradation process strongly affected the polysaccharide structure. It was found that the corresponding polysaccharide fingerprint peaks located between 890 and 960 cm^{-1} disappeared. The intensity of the peak located at 1038 cm^{-1}, attributed to the C-O-C group, decreased significantly pointing out the scission of the chitosan chain in agreement with previous reports [35].

For all the samples prepared with G, the peak related to the carbonyl group of lactic acid disappeared suggesting the existence of quick degradation zones.

In addition, there was a significant decrease of absorbance in the band corresponding to hydroxyl groups located between 3000 and 3600 cm^{-1} (Figures 8-10).

Conclusion

Biodegradable films based on chitosan/glycerol blends were obtained by solvent-casting evaporation method. The effect of film composition on the morphological characteristics, thermal and mechanical properties, swelling degree, water vapor permeability and degradation in soil was investigated.

From the results obtained in this work, it was demonstrated that films properties can be tailored by changing the glycerol composition.

Intermolecular interactions between chitosan chains were greatly affected by glycerol content due to a cluster effect produced by the glycerol molecules surrounding these chains.

Taking into account the potential use of the obtained films in the treatment of skin injuries, the mechanical properties, swelling degree and water vapor permeability are favored from the addition of high amounts of glycerol.

Acknowledgement

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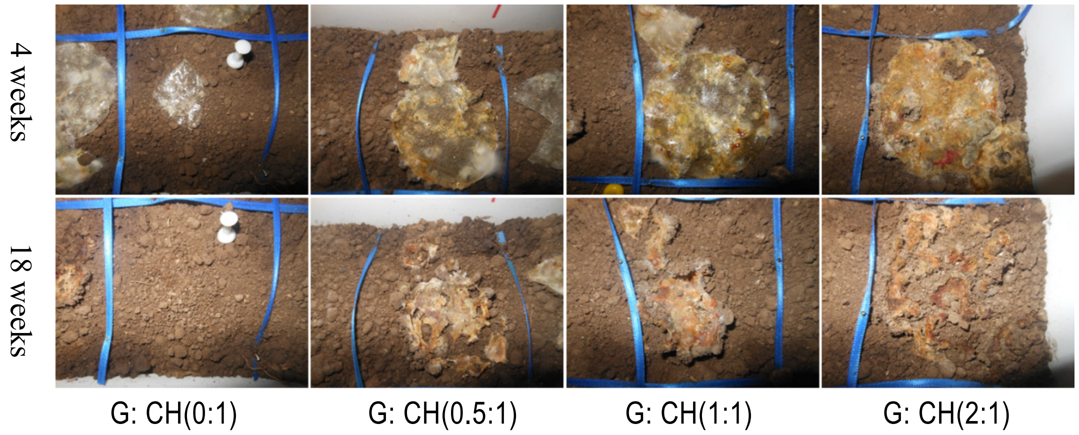

Figure 9: Films degraded in soil: optical micrographs after 4 and 18 weeks.

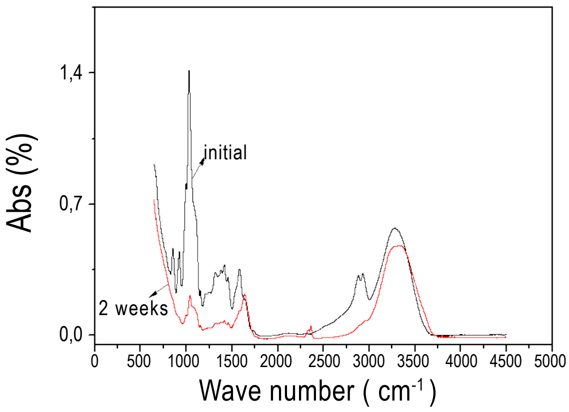

Figure 10: Evolution of FTIR spectra for a film prepared with G:CH (2:1) at initial time and after 2 weeks.

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