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Differences in the interaction of water with starch and chitosan films as revealed by infrared spectroscopy and differential scanning calorimetry

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Abstract

A comparison of starch and chitosan, with respect to the state of absorbed water at low water content was carried out using infrared spectroscopy and differential scanning calorimetry (DSC). The infrared water deformation band appears as a simple, symmetric band at 1647 cm^{-1} in the case of starch while it is a doublet band (1647–1582 cm^{-1}) for chitosan. The band splitting observed for chitosan would indicate a stronger interaction of water molecules with the hydroxyl group than with the amine group. The observed DSC endotherm is more symmetric for chitosan than for starch samples equilibrated with air. The differences observed between the thermograms of the two polymers are explained by means of a molecular mechanism of water removal, upon heating, based on the different strengths of the interaction of water with hydroxyl and amine groups.

Keywords: Infrared spectroscopy; Differential scanning calorimetry; Thermograms

1. Introduction

There is an increasing interest in the production of novel materials from renewable resources (Fritz et al., 1994). Natural polymers are replacing synthetic polymers in different applications (Piskin, 1994; Rathke & Hudson, 1994) partly because the waste from natural polymers is normally biodegradable (Ehring, 1992). Packaging is one potential area for the introduction of biomaterials, particularly starch (Fritz et al., 1994). Chitosan is the *N*-deacetylated derivative of chitin, a natural polysaccharide widely found in crustaceans and insects (Rathke & Hudson, 1994). By varying the degree of deacetylation the amount of amine groups can be altered. As a consequence chitosan has potential for ion exchange applications because of the complexation ability of the amine group (Muzzarelli, 1973).

Starch and chitosan are hydrophilic and retain considerable amount of water. The amount depends on the relative humidity, but in most cases is above 10%, in contrast to the small amount (about 1%) observed for most common synthetic polymers (Roff & Scott, 1971). Thus, the infrared spectra of starch and chitosan are greatly affected by the presence of absorption bands related to water. Direct evidence for this can be obtained from the analysis of difference spectra calculated from two FTIR spectra (Konig, 1975; Konig & Antoon, 1978) recorded from the same film sample at different water contents, i.e. usually with reference to a dried sample (Rueda, Viksne, Kajacks, Baltá-Calleja, & Zachmann, 1995).

It is known that because of associations through hydrogen bonding most polysaccharides do not melt but degrade upon heating above a given temperature. For temperatures below that of polymer degradation, their thermograms show a very broad endotherm which is related to the water evaporation process (Bershtein & Egorov, 1994). The use of correctly sealed DSC pans allows structural information about both the polymer (Ratto, Hatakeyama, & Blumstein, 1995) and the type of water present in the polysaccharide to be obtained (Bershtein & Egorov, 1994; Ratto et al., 1995). The influence of moisture on the glass transition temperature in relation to brittleness on ageing observed in starch has been studied by DSC calorimetry (Shogren, 1992).

The aim of this work is to highlight the differences observed in the infrared spectra and in the shape of DSC traces between samples of starch and chitosan. In particular the doublet structure of the water deformation vibration band observed in the case of chitosan is contrasted with the single, symmetric band observed for starch. To explain the differences in DSC traces between starch and chitosan a molecular mechanism of water removal upon heating is

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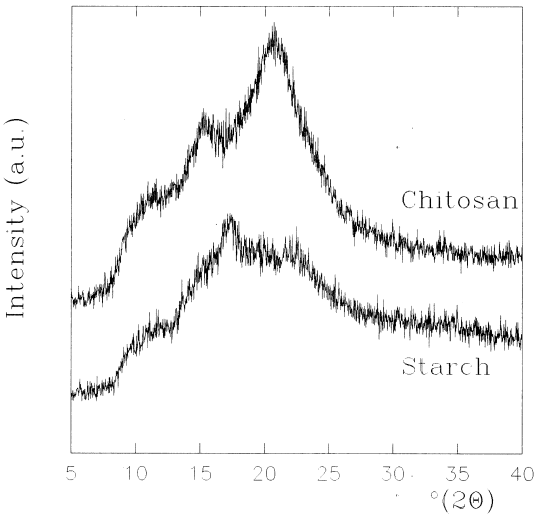


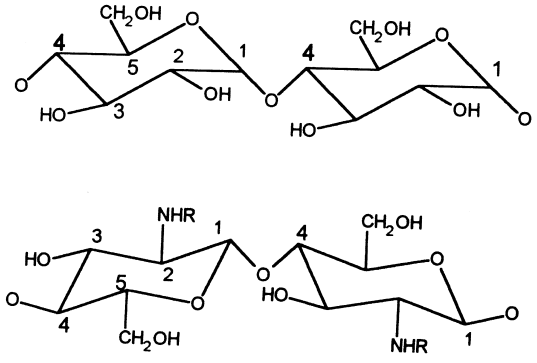
Fig. 1. Wide angle X-ray diffractograms of starch and chitosan thin films.

proposed. This molecular mechanism takes into account the different interaction of water with the amine and the hydroxyl groups.

2. Experimental

2.1. Materials

The chemical structure of the disaccharide repeat unit in starch and chitosan is schematically shown:



where R is H (amine group) or $-COCH_3$ (acetylated amine group).

The main differences between starch and chitosan are the glucoside linkage: α(1,4) for starch and β(1,4) for chitosan. Further, the hydroxyl group of the second carbon is replaced by the amine group which appears acetylated in the case of natural polymer chitin. Chitosan is obtained by partial deacetylation of chitin. Chitosan samples were obtained from Bioquimica Austral, Chile, with a claimed degree of deacetylation of 86%.

2.2. Solution cast films: characterization

A mixture of water and potato starch (2% in weight) was heated at 70°C under stirring till transparent. After 20 min this solution was transferred to a horizontal Petri dish, filled to about 4 mm in height, and stored at room temperature allowing it to dry. Thus, a very thin starch film (17 μm) and thicker ones (30–40 μm) were obtained. The preparation of chitosan films have been described previously (Nogales, Ezquerra, Rueda, Martínez, & Retuert, 1997). A 19 μm film was used in this study.

X-ray diffractograms of the film samples (five piled up pieces of polymer film) were recorded by means of a Seifert XRD-3000 θ/θ diffractometer (Fig. 1). Ni filtered CuKα radiation (40 kV, 35 mA) and a recording speed of about 1°(2θ)/min (0.03°(θ) every 2 s) were used. Each diffractogram shows distinct broad, intensity maxima revealing the semicrystalline character of the solution cast films. The broad maximum observed at about 11° comes out from the sample holder. Density of air stored films was measured by a flotation method using a carbon tetrachloride/dioxan mixture. Macroscopic density values of 1.49 and 1.42 g/cm^3 were found for starch and chitosan films, respectively. Thus, an accurate measurement of the thickness of the films used for infrared spectroscopy was determined by weighing a given area (3–4 cm^2) of the film sample. A Sartorius balance with an accuracy of 10^{-5} g was used throughout.

2.3. Infrared spectroscopy

Transmission infrared spectra of the films were recorded, at room temperature, using a Perkin–Elmer FTIR spectrometer (model 1725) with a resolution of 2 cm^{-1} after six accumulated scans. The film was mounted in the sample holder of an infrared cell SPECAC which was used to obtain spectra of the film. The film, inside the infrared cell, was dried by applying rotary pump vacuum, using a rotary pump, for at least one day. The real time spectra for a water sorption experiment were recorded after exposing the sample holder with the dried film to ambient air. The film sample remains inside the infrared cell for the time required for the spectrum recording only (Rueda et al., 1995). A hydrated sample of the same film was obtained after storage of the sample holder inside a closed glass vessel, where the film was held 1 cm above the level of water for 2–3 days. Spectra during water desorption experiment were obtained from an initially hydrated sample which had been exposed to ambient humidities (~ 20–33%).

2.4. Differential scanning calorimetry

Aluminium pans for DSC measurements were filled by piling up several circular pieces cut from the polymer film in order to use about 10 mg of sample. DSC scans in the range of 3°C up to 250°C were obtained using a Perkin–Elmer DSC-4 calorimeter at a heating rate of 10 K/min and a cooling rate of 320 K/min. A cooling system allowed DSC scans to be obtained just above the water melting point. The calorimeter is also equipped with a dry glove box, allowing samples to be handled under dried nitrogen during the experiments. There is a sample elevator to facilitate the

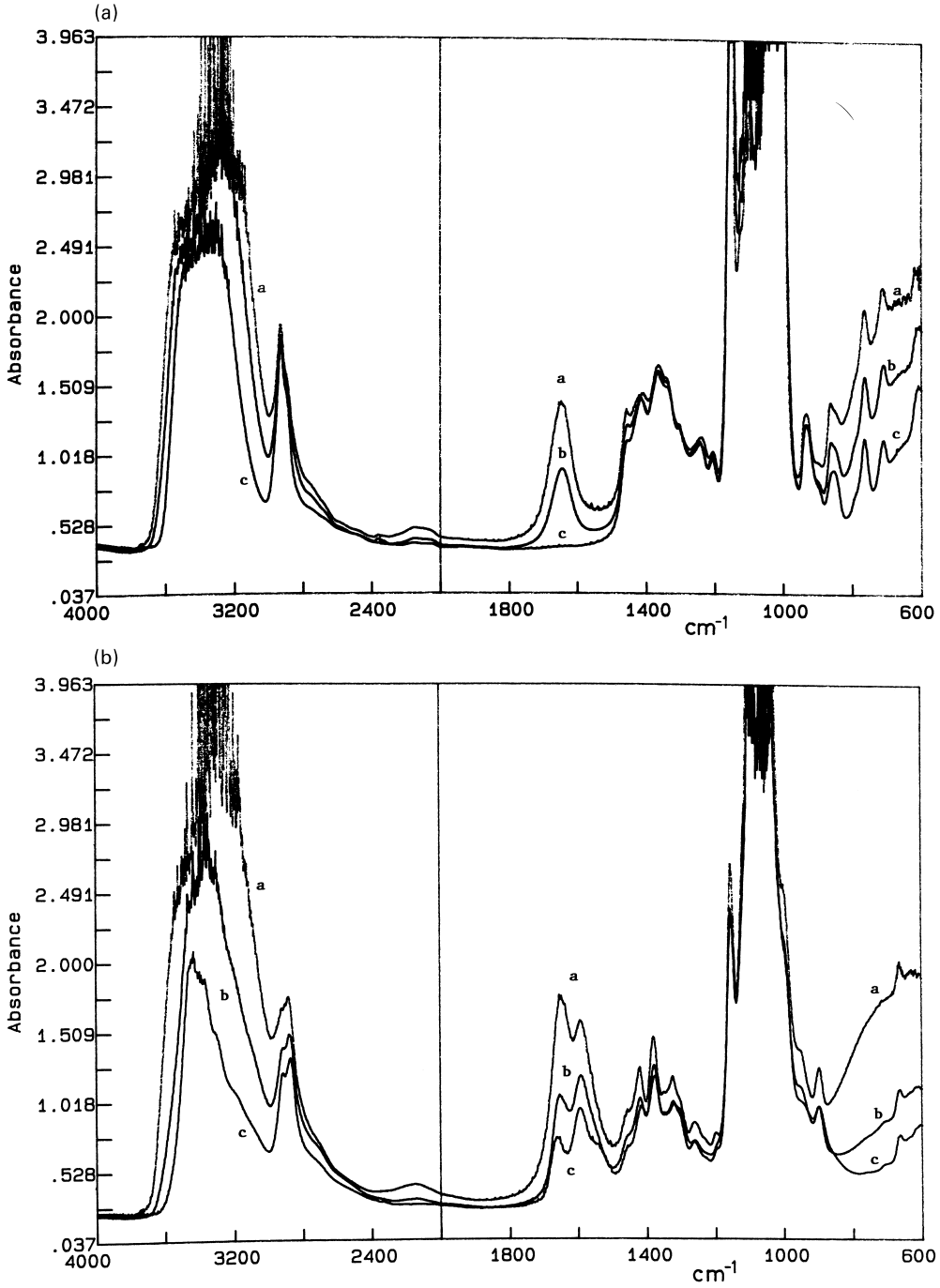


Fig. 2. (a) Transmission infrared spectra of a starch film (17 μm thick) with different water concentrations: a; hydrated. b; air stored, and c; dried. (b) Transmission infrared spectra of a chitosan film (19 μm thick) with different water concentrations: (a) hydrated; (b) air stored; and (c) dried.

operation of taking the sample out, therefore lowering the elapsed time before weighing the sample after DSC recording.

3. Results

3.1. Influence of water content on the infrared spectra

Starch and chitosan films stored in air (room humidity about 25%) absorb about 11–14% water relative to the weight of the dried film. This amount of water reaches values up to 31%, for starch, and 38% for chitosan when exposed to humidities close to 100%. Fig. 2(a) shows the mid-infrared transmission spectra obtained from the same starch film (17 μm thick) under different hydration conditions: equilibration in air (spectrum b), hydrated (spectrum a) and dried (spectrum c) films. We observe large intensity variation of the very strong band at about 3400 cm^{-1}

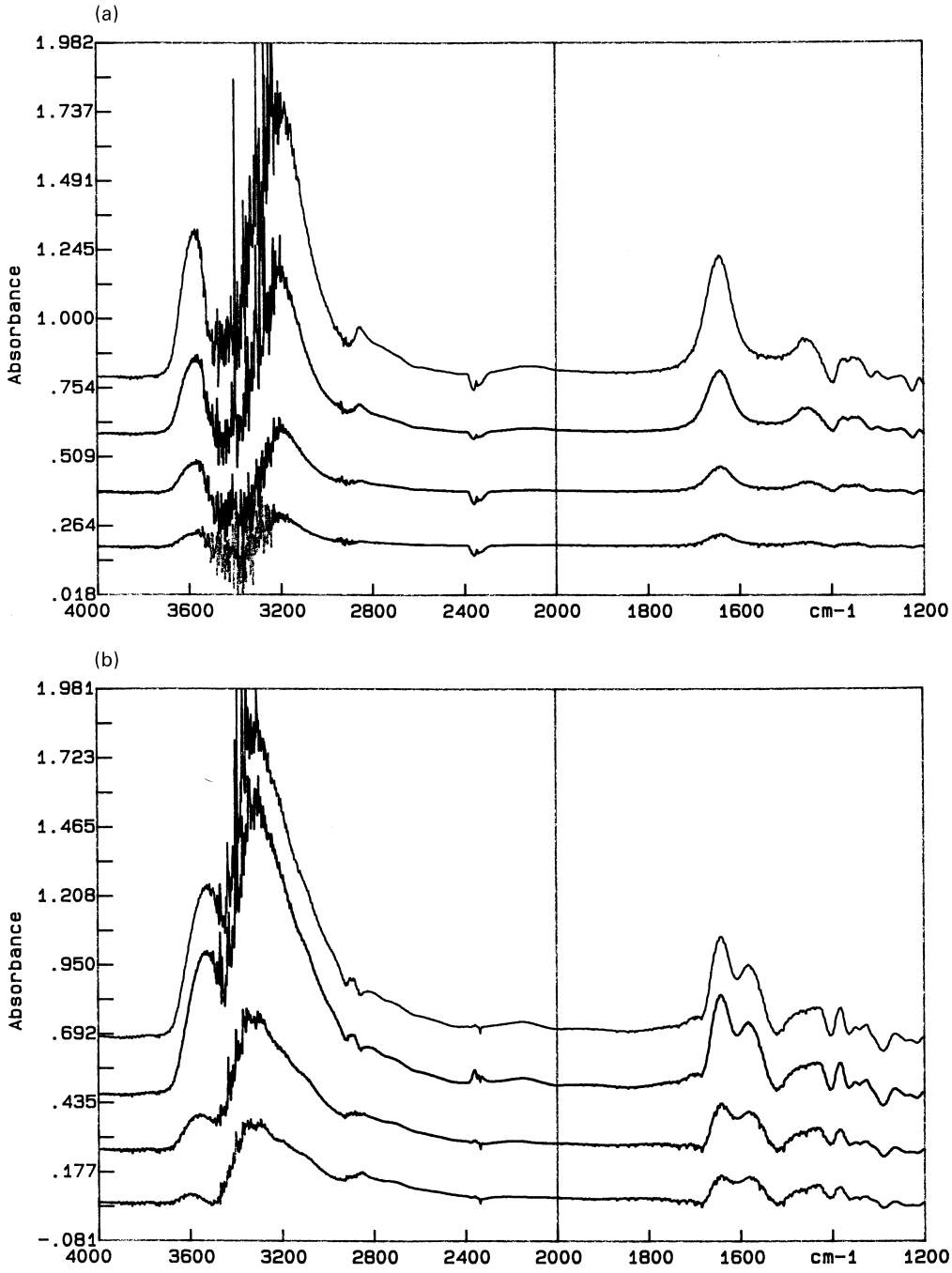


Fig. 3. The 4000–1200 cm^{-1} spectral region of difference spectra for a water sorption process using as reference a vacuum dried film of starch (a) and chitosan (b) exposed to air. Sorption time values (from bottom to top): (a): 2, 10, 45 and 271 min; (b): 1, 8, 60 and 375 min.

associated with the OH stretching vibration mode of alcohol groups (Bellamy, 1954). The broad character of the band observed even for a dried film would indicate a strong interaction between hydroxyl groups (Thompson, Nicholson, & Short, 1950). For air stored and hydrated starch films this absorption becomes broader because of the interaction of polar groups with the water molecules incorporated in the polysaccharide film. In addition, a more dramatic change occurs for the single, symmetric band at 1647 cm^{-1} associated with the deformation vibration mode of OH bond from water molecules (Bellamy, 1954). Spectrum c clearly indicates that water molecules can be completely removed by prolonged evacuation times (one day) under a rotary pump vacuum.

As for starch the infrared spectra obtained from a chitosan film (19 μm thick) are shown in Fig. 2(b). The main differences between starch and chitosan spectra are in the 1600 cm^{-1} region because of the appearance of polymer

Table 1
Variation of frequency position (ν), peak width at half maximum intensity, peak area and relative peak area for the two main components of the water deformation band during a water sorption process of a dried chitosan film exposed to air

t (min)	ν (cm^{-1})	Width (cm^{-1})	Area (cm^{-1})	Relative area (%)
< 2	1581.8	63.1	7.1	57.8
	1644.2	47.3	5.2	42.2
8	1582.7	62.5	9.5	55.8
	1644.2	43.9	7.5	44.2
11	1582.7	62.1	10.4	54.6
	1644.2	43.7	8.7	45.4
20	1582.7	61.1	12.6	52.8
	1644.1	43.4	11.2	47.2
28	1582.2	59.3	13.5	49.4[a]
	1644.0	44.4	13.2	48.5
375	1580.9	56.0	15.0	44.9[a]
	1644.2	46.5	17.1	51.3
Chitosan	1582.1	58.9	21.0	44.7[a]
	1647.6	49.3	24.1	50.7

[a] Calculated with a third peak component.

bands in the case of chitosan, which obviously remain after removal of water (spectrum c of Fig. 2(b)). The spectrum c clearly shows a triplet band. Two of them (at 1622 and 1542 cm^{-1}) can be associated with the amide I and amide II bands of the acetamide group (present in 14% of the glucoside rings). The more intense peak at 1596 cm^{-1} can be related to the deformation vibration mode of the NH bond of the amine group (present in 86% of the glucoside rings) (Bellamy, 1954).

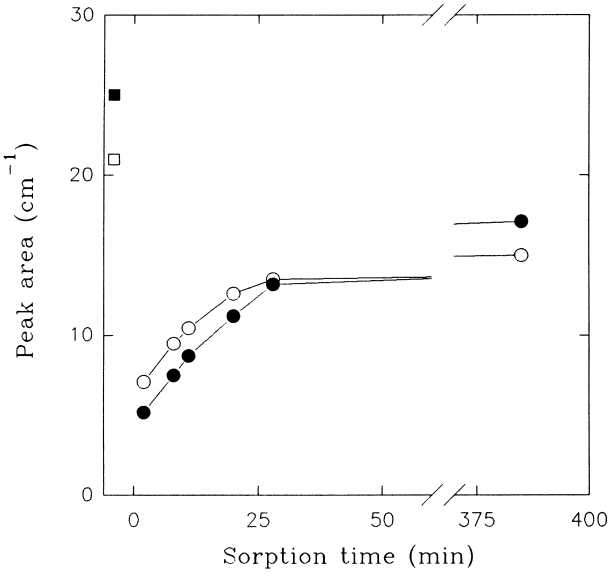


Fig. 4. Variation of the calculated absorbance of the two component peaks at 1582 cm^{-1} (open symbols) and 1644 cm^{-1} (filled symbols) when a dried chitosan film is exposed to air.

3.2. Variation of the water deformation band during water sorption/desorption experiments

The water contribution to the infrared spectra shown in Fig. 2(a) and (b) was investigated from the calculated difference spectra. These were obtained taking as reference the spectrum for the dried sample (spectrum c of Fig. 2(a) and (b)). Fig. 3(a) and (b) represents the 4000–1200 cm^{-1} spectral region of some difference spectra corresponding to a water sorption process for starch (a) and chitosan (b) films. These difference spectra would mainly represent the infrared spectrum of water. However, the presence of water in the polymer film could also affect both frequency position and the intensity of vibration modes, particularly, of polar groups in relation to the spectrum of a dried film. As a result of this, additional positive or negative "bands" can be observed with increasing water content in the difference spectra of Fig. 3(a) and (b). The broad stretching band (around 3100 cm^{-1}) is greatly perturbed in its central frequency interval. This is due to the loss of instrumental sensitivity because of the high intensity of the subtracted polymer band. Consequently, the stretching region is not accurate for quantitative analysis purposes. We will therefore focus our attention on the weaker band around 1600 cm^{-1} which corresponds to the deformation vibration mode of OH from water. While the spectra derived from starch samples show a single, symmetric band at 1647 cm^{-1} those for chitosan revealed a doublet structure. Further, a change in the relative intensity of the two components bands occurs over the water sorption time.

The variation of the doublet bands with sorption time was analysed by means of a peak fitting program. Results regarding peak components (Gaussian type) are collected in Table 1. The frequency position of components remains nearly constant and a slight decrease of the width of both peaks is observed. In the last two columns of Table 1 the peak area (cm^{-1}), and the relative peak area, in percent, of the two peak components are presented. For the longer sorption times and for air stored chitosan the total area is less than 100 because a third peak component around 1710 cm^{-1} (see Fig. 3(b)) was considered for fitting. For a better description of the results in Table 1 the variation of the peak area of the two band components are plotted as a function of sorption time in Fig. 4. Data for the same film sample before drying (squares) are also included for comparison with the levelling off values observed after about 6 h.

Fig. 5 shows the variation of the reduced integrated absorbance (in cm^{-2}) of the water deformation band with exposure time to air for a hydrated (filled symbols) and a dried (open symbols) sample of starch (triangles) and chitosan (circles). As samples are very thin films, both water sorption/desorption processes occur in relatively short periods of time (one day). In addition, a very large influence of the room humidity on the water sorption/desorption processes prevented us doing a comparison of the water sorption kinetics between the two polymers. The time evolution

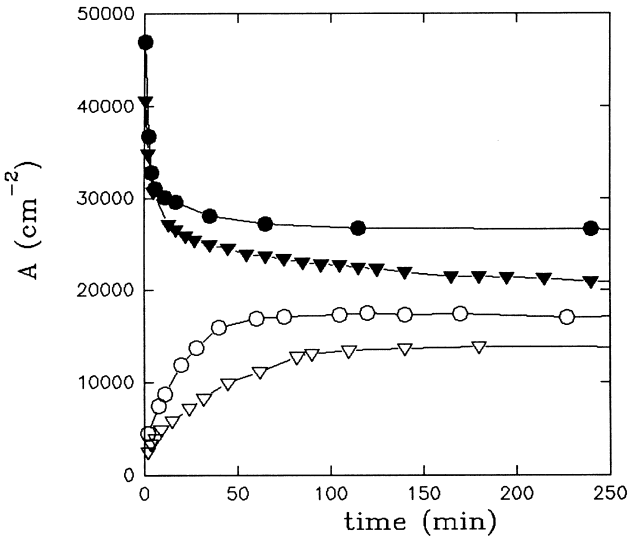


Fig. 5. Time evolution of the reduced absorbance of the water deformation band measured from difference spectra for a water desorption (filled symbols) and a water sorption (open symbols) process carried out with starch (triangles) and chitosan (circles) films.

shown for the water deformation band have similarities to that reported for the water stretching band during similar sorption/desorption processes carried out on hydrophobic polymers (Rueda et al., 1995; Rueda & Varkalis, 1995).

It is noteworthy that the quantitative analysis of the deformation band (using reduced integrated absorbances measured in the 1790–1520 cm^{-1} interval) for starch and chitosan samples revealed a similar band absorptivity: $K_{1790-1520} = 1.1 \times 10^5\,cm^{-2} = 2 \times 10^6\,cm\,mol^{-1}$. This value is about one order of magnitude less than that observed for the stretching band of water in organic solvents (Paul & Ford, 1982) and hydrophobic polymers (Rueda et al., 1995).

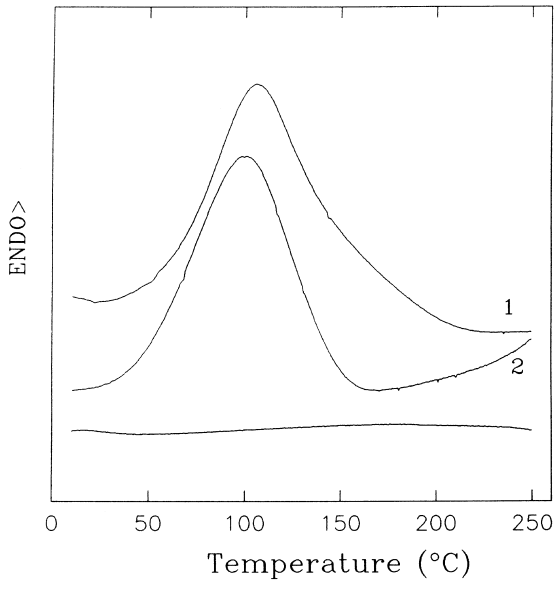


Fig. 6. First DSC traces for starch (1) and chitosan (2) films. The trace obtained on a rerun for starch is also shown.

Table 2
Temperature of peak maximum, peak width at half intensity maximum and relative peak area values found for the two peak components which fit the observed endotherm from a starch film

Peak component	T_{max} (°C)	Width (°C)	Peak area (%)
1	94.4	56.9	65.3
2	147.3	68.0	34.7

3.3. DSC endotherms with reference to water content

Fig. 6 shows the first DSC traces from the starch and chitosan film samples stored in air. The temperature range from 3°C up to 250°C was selected for two reasons: (1) to avoid endothermic signals related to the melting of frozen water around 0°C and (2) to limit possible sample degradation. For higher temperatures, in the case of starch the broad endotherm is accompanied by a sharp endothermic curve above 270°C due to a degradation of the sample. The observed endotherms are related to the evaporation of water present in the samples (Bershtein & Egorov, 1994) that occur over a large temperature interval (about 100°C for chitosan and 150°C for starch). For chitosan the endotherm maximum, centred about 100°C, is shifted down by some degree in relation to that of starch. For starch an asymmetry of the endotherm from the high temperature side is observed. By means of a fitting curve program two Gaussian peak components can fit the observed thermogram of starch. Values for the temperature of the peak maximum, the peak width measured at half intensity maximum and the relative area of the two peak components are given in Table 2. A second DSC run, immediately following the first one yielded an horizontal DSC trace (Fig. 6). This supports the view that water evaporation occurs during the first DSC run.

To compare the thermal behaviour of water present in starch and chitosan films, the observed endothermic area of a first DSC run was correlated to the water content of the sample. To obtain the latter we have measured the loss of weight of the sample (encapsulated in a DSC pan) immediately after carrying out the DSC run and determined the relative water content of the sample. Some of the encapsulated samples were used again to obtain a further, second DSC trace after different storage time values (from one day till several weeks), i.e. when the sample takes up a given amount of water. In Fig. 7 we have represented the endotherm peak area against the relative water content for starch (open symbols) and chitosan (filled symbols) samples. A similar, linear relationship is obtained for both the polysaccharides. An average slope of about 550 cal/g (41 kJ/mol) is derived which is similar to that accepted as the enthalpy of vaporization of water (Lide, 1995).

4. Discussion

Comparing the difference-spectra of Fig. 3(a) (from starch) and Fig. 3(b) (from chitosan), which mainly show

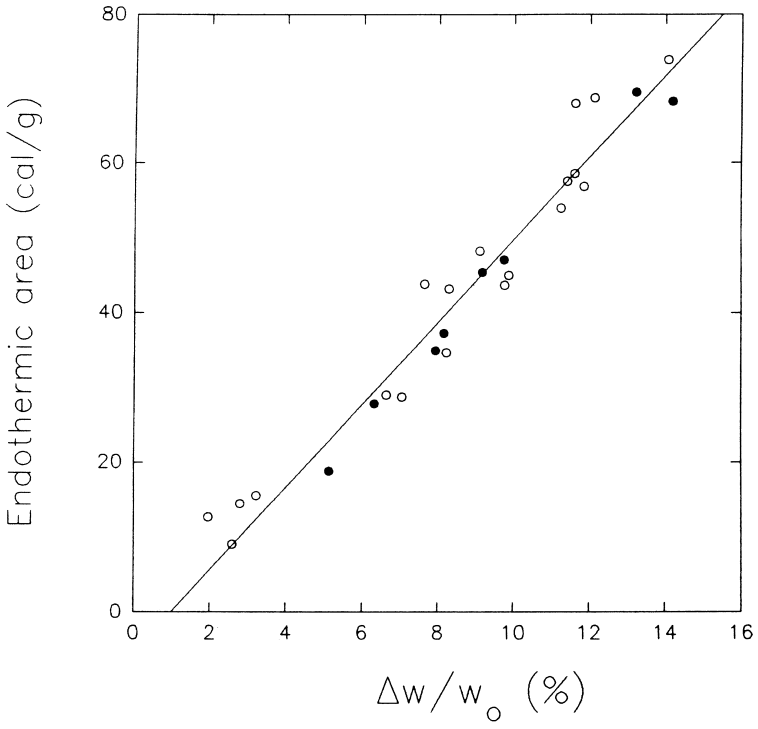


Fig. 7. Variation of the endothermic peak area with the relative water content for starch (open symbols) and chitosan (filled symbols) samples.

absorption bands related to water, we must ask about the reason for the doublet structure of the water deformation band observed for chitosan (Fig. 3(b)) in contrast to the single band observed for starch. Firstly, considering the chemical structure of both polymers, one could relate the band splitting to the presence of water molecules linked to the two polar groups (hydroxyl and amine groups) present in chitosan. Indeed, the higher frequency peak appears at the same frequency position (1647 cm^{-1}) observed for the single band of starch which contains only hydroxyl groups. It can therefore be deduced that the lower frequency peak at 1582 cm^{-1} is related to water bound to amine groups. From the relative frequency position of the two bands it can be said that the interaction of water with hydroxyl groups is stronger than with amine groups. Secondly, because of the changes in both, intensity and frequency position of some vibration modes from polymer polar groups interacting with water it is also possible that the low frequency band at 1582 cm^{-1} observed for chitosan spectra (Fig. 3(b)) corresponds to N–H vibration mode perturbed by hydration. Nevertheless, it is worth noting that a doublet band, with similar band structure and frequency values, was also observed in the difference spectra for other chitosan film samples with a much lower degree of deacetylation (about 28% and 53%) (Rueda & Martínez, unpublished results). Therefore, because of this it seems that the first interpretation for the doublet structure of the water deformation band for chitosan is more acceptable than the second one mentioned earlier.

The results shown in Table 1 and Fig. 4 would indicate that the relative amount of water molecules linked to the two polar groups of chitosan changes during the water sorption process. Indeed, for low water content sample (small sorption times) the intensity of the band at 1582 cm^{-1} is larger than that at 1647 cm^{-1}. Later, the area of the band at 1647 cm^{-1} surpasses that of the band at 1582 cm^{-1}.

The thermograms of Fig. 6 would indicate that water molecules are removed over a narrower temperature interval for chitosan compared to starch. In the case of starch, taking into account the asymmetry of the endotherm, it could be said that the removal of a considerable amount of water molecules (about one-third of them (Table 2) is delayed. We could say that the residual molecules of water in starch behave as they were more strongly bound to the polymer than those first removed.

4.1. Mechanism for removal of residual water upon heating

The water content for air stored samples (11–14%), expressed in molecular terms, is about one molecule of water per monomer unit. The thermograms obtained here (Fig. 6) mainly describe the thermal behaviour of water molecules in terms of their evaporation from the polymer sample as a function of temperature. Therefore, the endotherm shape and temperature position will provide information about the water molecules removed from the polymer at the heating rate used.

The comparison of endotherms shown in Fig. 6 suggests that the interaction of water with the polymer is stronger for starch than for chitosan. Thus, in the case of starch the

removal of water occurs up to temperatures about 220°C while in the case of chitosan only up to around 160°C.

What is the reason for this? As the starch and chitosan films investigated here are semicrystalline materials (Fig. 1) the expected differences in mobilities of water molecules through ordered and disordered regions (Peterlin, 1957; Rueda & Varkalis, 1995) could not be exclusively invoked to explain the asymmetry observed for starch as compared to chitosan.

According to infrared results (splitting of the water deformation band, Fig. 3(a) and (b)) water is bound to the hydroxyl group more strongly than to the amine group. Consequently, it might be thought that in the case of chitosan the water molecules linked to amine groups could be removed more easily (at lower temperatures) than those molecules linked to the hydroxyl groups. This could explain the lower temperature for the endotherm maximum of chitosan compared to starch (Fig. 6). However, no asymmetry was shown by the chitosan endotherms.

We think that the narrower and more symmetric endotherm observed for chitosan is due just to the coexistence of amine and hydroxyl groups in the glucoside ring. In this case, because of the different strength of the association of water to hydroxyl and amine groups the release of water molecules could preferentially occur via the amine group. Upon heating, the thermally activated water molecules will tend to release and molecules which are bound to amine groups will release earlier than those bound to hydroxyl groups. Thus, it could be said that amine groups are facilitating the release of water molecules from chitosan. In the case of starch, the water molecules which only interact with hydroxyl groups will require a higher thermal activation energy to be released from the glucoside ring. At temperatures above the endotherm maximum the polymer film becomes increasingly lower in water content. The residual molecules of water diffusing through the polymer film will be trapped in glucoside rings free of water. Therefore, the DSC trace for starch is shifted towards higher temperatures even showing an asymmetry at the upper temperature side (Fig. 6).

5. Conclusions

- Starch and chitosan films stored in air (room humidity about 25%) absorb significant amount of water (11–14 wt.%). This relative amount of water reaches values up to 31 wt.% for starch, and 38 wt.% for chitosan when exposed to humidities close to 100%.
- In the case of chitosan the water deformation band splits into two bands at 1647 and 1582 cm^{-1} which are related to water bound to hydroxyl and amine groups, respectively. Such a splitting of the band would indicate that the interaction of water molecules with the amine group is weaker than with the hydroxyl one.
- A common, linear relationship between the endothermic peak area and the relative water content of the samples was found for starch and chitosan films. A similar infrared absorptivity ($K_{1790-1520} = 2 \times 10^6$ cm mol^{-1}) for the deformation vibration mode of the water present in starch and chitosan films was observed.
- It is proposed that the removal of water, upon heating, preferentially occurs via amine group in the case of chitosan. As a consequence, the temperature of the endotherm maximum is lower for chitosan than for starch. As far as endotherm shape is concerned, the presence of amine groups in the glucoside rings of chitosan facilitates the evaporation of water giving rise to more symmetric endotherms in contrast to the asymmetric endotherms of starch.

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