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Effect of Cross-Linking with Calcium Ions on the Physical Properties of Alginate Films

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Three films of sodium alginate, with different amounts of guluronic fraction, were investigated with different techniques. On increasing the fraction of guluronic units the chain-to-chain interaction was promoted. The three samples were ionically cross-linked with calcium ions by soaking the films in a solution of calcium chloride. The introduction of the cross-linking points caused an appreciable change in the physical properties, and the results were discussed in terms of different composition of the materials and in terms of the increased free volume during the cross-linking process.

Introduction

In recent years the use of natural polymers coming from renewable sources has greatly increased. This is mainly due to the high postconsumer plastic waste produced every year and the reduced availability of landfills. Biodegradable polymers, known for many decades but ignored mainly because of the low cost of synthetic polymers, could replace synthetic polymers in many applications, reducing in this way the problems of disposability of traditional plastics and the dependence on petroleum.[1−5]

Alginates are linear water-soluble polysaccharides comprising (1−4)-linked units of α-D-mannuronate (M) and β-L-guluronate (G) at different proportions and different distributions in the chain.[6,7] They are present in brown algae and can also be found in metabolic products of some bacteria.[8] The chemical composition and sequence of the M and G residues depend on the biological source and the state of maturation of the plant.[9] The alginates, as well as all the polysaccharides, are polydispersed in terms of molecular mass so that they are more similar to the synthetic polymers than to other biopolymers such as proteins and nucleic acids.

Alginates are well-known natural ionic polysaccharides used mainly as food additives, thickeners, gelling agents, and in the controlled delivery of drugs.[10−12]

The great interest with this family of polysaccharides is strictly related to the gelling properties. Alginate solutions can form gels either by lowering the pH below the pK_a value of the uronic residue or in presence of divalent ions. The divalent ions cooperatively interact with blocks of guluronic units to form ionic bridges between different chains.[10,13] The ability to form this kind of interaction depends on the length of the G-blocks. The most popular model to account for the chain-to-chain association is the "egg box model",[14,15] although further work is still in progress on this topic.[16−19] In this model the G-blocks form a three-dimensional arrangement in which Ca ions are embodied in cavities like eggs in a cardboard egg box. According to some authors,[16−19] it seems that the Ca ion can be localized in different special arrangements, still promoting the lateral association. The affinity of alginate chains with respect to divalent ions decreases in the following order: Pb > Cu > Cd > Ba > Sr > Ca > Co, Ni, Zn > Mn.[20] The ion Mg^{2+} does not promote gelation.[21]

The alginate can be considered a block copolymer containing three types of blocks (GG, MM, MG). The stiffness of the three blocks decreases in the order GG > MM > MG.[22] The physical and chemical properties of alginates strictly depend on their composition and the sequence of guluronic and mannuronic residues in the polymeric chain.[13]

In this study we analyze three different alginates with different molecular parameters with respect to the different ability to ionically cross-link with calcium ions.

Experimental Section

Materials and Film Preparation. Two samples of sodium alginate were kindly supplied by FMC BioPolymer, Belgium: Protonal LF 20 with high guluronic acid content and Scogin LV with lower guluronic acid content; the third sample of sodium alginate was supplied by Sigma-Aldrich.

The films were prepared by casting from aqueous solutions. Solutions at 1% of the three polymers were prepared, while stirring, at the temperature of 70−75 °C. All solutions were filtered and kept for a few minutes under vacuum. After this treatment the solutions were poured, avoiding bubble formation, into a polyester mold kept in plane to ensure the homogeneous thickness of the films. The films thicknesses were in the range of 40 μm. All films were allowed to form during exposure to the atmosphere for 3 days. Prior to testing, the films were equilibrated at 45% relative humidity by storing them in a desiccator over a saturated solution of calcium nitrate at room temperature.

The cross-linked samples were prepared by soaking for 30 min the films in a 2% w/v aqueous solution of calcium chloride.

Molecular Characterization. Alginates, like other polysaccharides, are polydispersed with respect to the molecular mass and have different contents of guluronic and mannuronic moieties. The concentration of G and M residues and the molecular masses were determined by NMR and SEC (size exclusion chromatography).

The chemical composition and the sequential structure of sodium alginate were determined by ^{1}H nuclear magnetic resonance spectroscopy. The NMR methodology and the assignments of peaks were based on data published by Grasdalen, Heyraud and co-workers.[6,23−25] The analysis was performed using a 500 MHz Varian Unity INOVA spectrometer. The spectra were collected at 50 °C following the

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Table 1. Molecular Parameters

sample	%G	M_n (Da)	M_w (Da)	M_w/M_n
A	37 ± 1	2.3×10^5	1.2×10^6	5.2
B	45 ± 1	1.1×10^5	5.9×10^5	5.4
C	62 ± 1	2.0×10^5	5.1×10^6	5.5

Standard Test Method F 2259-03.[26] In order to know the chemical compositions of the three investigated polysaccharides, the monad frequencies F_M (fraction of mannuronate residues) and F_G (fraction of guluronate residues) were found out by numerical elaborations of spectra data relative to the integrated intensities of specific signals in the range of 5.5−4.5 ppm of the ^{1}H NMR spectra.

The average molecular masses, M_W, and the distribution of the molecular masses in the alginate samples were determined by SEC. Three columns, HB 40, HB 1000, and HB 40 from PSS Mainz, a detector Shodex RI 71, Fa. Showa Denko, and an SP Thermo Separation Products pump (TSP) were used.

The calibration was performed using dextran; the eluent was a 0.1 M solution of $NaNO_3$ at a flow rate of 1 mL/min.

The molecular parameters of samples A, B, and C are reported in Table 1. From the data of Table 1 we notice that the three samples are similar in terms of the molecular mass. This is particularly interesting since all the differences in some properties can be ascribed to the different concentration of guluronic residue.

Thermogravimetric Analysis. Dynamic thermogravimetric analysis was carried out with a Mettler thermogravimetric analyzer model TG 50. The measurements were performed on samples of about 8−10 mg, placed in ceramic crucibles, from 40−400 °C at a heating rate of 10 °C/min, under nitrogen atmosphere with a nominal gas flow rate of 25 mL/min.

Differential Scanning Calorimetry (DSC). DSC measurements were carried out on samples of 7−8 mg over the temperature range of 0−400 °C using a Mettler TA 3000 DSC purged with nitrogen and chilled with liquid nitrogen, with a heating rate of 10 °C/min. All the samples, before the run, were kept in the DSC for 2 h at 130 °C to allow the elimination of some water that would mask the T_g of alginates.

Dynamic-Mechanical Analysis (DMA). DMA tests were carried out on a Perkin-Elmer Pyris Diamond DMA in tensile mode at a frequency of 1 Hz in the range of −100 to 250 °C, under nitrogen atmosphere, at a heating rate of 3 °C/min. The heating rate was reduced to 1 °C/min for measurements in multifrequency. The frequencies used were 0.1, 0.5, 1, 2, 5, 10, and 20 Hz. The apparent activation energies, E_a, of the relaxations were calculated according to an Arrhenius-type equation, from the maximum values of the loss tangent at the seven frequencies. Also for these measurements, all the samples were kept at 130 °C for 2 h prior to every run.

Permeability. Water vapor permeability (WVP) was calculated with the following equation:

$$\mathrm{WVP} = \mathrm{WVTR}\ (L/\Delta p) \qquad (1)$$

where WVTR, expressed in g/m^2·s, is the water vapor transmission rate of films, L is the film thickness (m), and Δp is the water pressure difference between both sides of the film (Pa). WVTR was measured using the ASTM standard method E 96-00.[30] With this method WVTR was determined gravimetrically using a CEAST metal cup, with a metal disc which had a 3000 mm^2 exposed area for the test. The cup was filled with a small amount of anhydrous calcium chloride and placed in an environmental chamber set at 23 °C and 45% RH.

Results and Discussion

Swelling Phenomena. The thickness of the three samples was measured before and after the immersion in the solution of calcium chloride. The samples swelled and at the same time cross-linked. The thickness of swollen samples was measured after the drying and the conditioning at room temperature and 45% relative humidity. The thickness of the initial samples and the cross-linked ones are reported in Table 2. The thickness does not return to the original values since the cross-linking points, introduced in the swollen state, stabilize the conformations of this state. Moreover, on increasing the amount of G-units the thickness increases more.

Table 2. Change of Thickness as Effect of Cross-Linking

sample	thickness before cross-linking (μm)	thickness after cross-linking (μm)	thickness increase (%)
A	38	52	37
B	37	54	46
C	39	61	56

Table 3. Mass Loss at Different Temperatures

sample	mass loss at 100 °C (%)	mass loss at 150 °C (%)	mass loss at 180 °C (%)	onset decomposition (°C)
A	14.8	18.0	19.5	224
B	13.4	16.0	17.2	218
C	10.2	13.3	14.8	219
A−Ca	14.7	18.2	19.7	213
B−Ca	13.6	16.8	18.1	208
C−Ca	13.8	16.7	17.8	209

Thermogravimetric Analysis. In Figure 1 the mass loss of the three pure alginates and the alginates cross-linked with calcium ions are reported. In Table 3 the mass loss at specific temperatures and the temperature at which the decomposition process starts are reported. In the thermogravimetric curves we can distinguish three regions: the first from room temperature to 100 °C, the second between 100 and 200 °C, and the third, above 200 °C, where the decomposition occurs.

It has been reported by different authors that there are three kinds of absorbed water in hydrophilic polymers:[27−29] free, freezing bound, and nonfreezing water or bound water. Free water does not interact, via hydrogen bonding, with the polymeric chain. It behaves as pure water. The freezing bound water interacts only weakly with the polymeric chain, whereas the nonfreezing water is represented by molecules of water bound to the polymeric chain through hydrogen bonds. This kind of water does not show any first-order phase transition.

The mass loss in the first region can be ascribed to the elimination of free water. These molecules of water are the first molecules leaving the sample. Above 100 °C the samples still contain water. In fact, on increasing the temperature, the mass

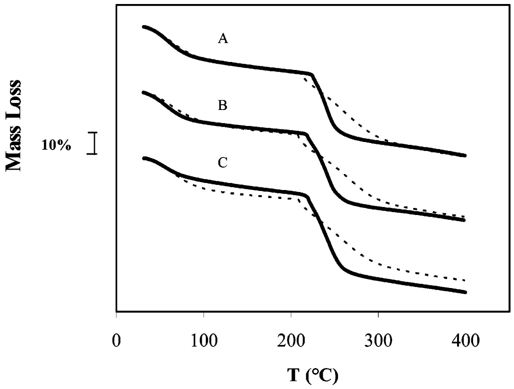

Figure 1. Thermogravimetric curves of samples A, B, and C (—) and samples A−Ca, B−Ca, and C−Ca (− − −).

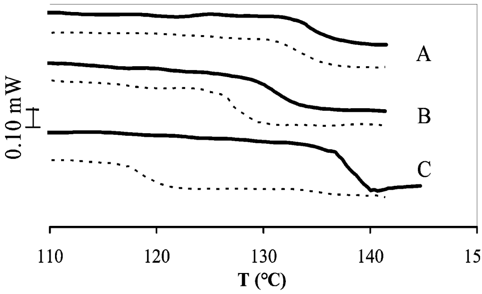

Figure 2. DSC scans of samples A, B, and C (—) and samples A−Ca, B−Ca, and C−Ca (− − −).

Table 4. Glass Transition, β Transition, Activation Energy, and Permeability

sample	T_g from DSC (°C)	T_β from DMTA (°C)	E_a (kJ/mol)	permeability ($ng{\cdot}s^{-1}{\cdot}m^{-1}{\cdot}Pa^{-1}$)
A	133	15	73	0.092
B	132	32	80	0.098
C	138	48	83	0.058
A−Ca	131	−13	75	0.133
B−Ca	127	−30	59	0.142
C−Ca	119	−35	68	0.137

of the samples still decreases, although the decomposition processes are not yet involved.

In our samples the loss of water is continuous in a wide interval of temperature, before the decomposition. This means that, in addition to the free water, the samples also contain the other two kinds of water.

From Figure 1 and Table 3, we notice that, on increasing the concentration of G-units, the water content decreases (less mass loss). This can be due to the fact that, on increasing the concentration of G-units, the possibility for the G-segments to interact with each other increases. In this way the number of groups, potentially interacting with the molecules of water, decreases. In the samples containing calcium ions, we notice that the quantity of water absorbed increases with respect to the not cross-linked samples. This can be related to the fact that the cross-linking process occurs in the swollen state, with the consequent increase of the free volume. In the swollen samples, a certain number of chain segments, before interacting with each other, are now available to interact, via hydrogen bonds, with additional molecules of water.

Another difference between the original and the cross-linked samples regards the degradation process. Usually cross-linked samples experience a delay in the degradation. In our case all the cross-linked samples behave in the opposite way. The lower temperature at which the degradation starts can still be related to the increased volume of samples containing calcium. The less packed chains are more vulnerable to the oxidation and, hence, to the degradation processes.

Differential Scanning Calorimetry. As for the thermogravimetric analysis, also for the DSC we have two regions of interest: the first in the region of 100−150 °C, in which the elimination of free water and the glass transition occur, and the second region, above 200 °C, at which the decomposition starts. The glass transition temperature, in samples containing free water, is not detectable. In fact, the endotherm related to the elimination of free water covers any signal related to the glass transition. For this reason, before each run, the samples were kept in the DSC at a temperature of 130 °C under nitrogen purge. After the elimination of free water the glass transition temperature is easily detectable. The DSC runs are reported in Figure 2, in the temperature range of 110−150 °C, and the data are summarized in Table 4. Sample C, as already reported in the literature, has the highest value of T_g due to the higher concentration of guluronic units. We notice that the T_g decreases as a result of the cross-linking. Usually, with the introduction of the cross-linking points, the T_g increases because of the reduced mobility of chain segments. In our case the cross-linking appreciably decreases the T_g, and the decrease becomes more pronounced on increasing the quantity of G-units. The increased mobility of chain segments in cross-linked samples can be explained in terms of increased free volume due to cross-linking in the swollen state. Since the decrease of T_g is more pronounced on increasing the content of G-units, we think that the free volume increases more on increasing the content of G. This hypothesis is also confirmed by the swelling data of Table 2. The cross-linking points represent a hindrance for the packing of chains, but at the same time, the chain segments between two consecutive cross-linking points experience an increased mobility because of the increase of the free volume.

In addition, from the thermogravimetric data, we notice that cross-linked samples contain more bound water that contributes to the lowering of the T_g.

Dynamic-Mechanical Analysis. The storage modulus (E') and the loss factor tan δ at 1 Hz for the three samples are reported in Figure 3 in a wide range of temperatures. In all the samples we notice a decrease of the storage modulus as a result of the cross-linking. This is unusual since the introduction of tie points between molecular chains reduces the mobility with a consequent increase of the modulus. In our case this anomalous behavior is, once again, related to the fact that the cross-linking process occurred in the swollen state. The introduction of cross-linking points in swollen samples represents a hindrance for the chains to recover the original packing. The decrease of the modulus can thus be related to an increase of the free volume and the consequent reduction of the chain-to-chain interaction.

More details can be found by analyzing the curves of tan δ. Two relaxations can be detected. The first is the glass transition (α transition) at temperatures above 100 °C, and the second (β transition) is a relaxation occurring at low temperatures. Unfortunately the T_g cannot be seen easily since it is disturbed by the onset of the decomposition process.

The β transition is a broad relaxation, and it is generally ascribed to the motion of short segments of chain or to lateral groups. For the alginates we do not have any support from the literature, but for systems like poly(vinyl alcohol) the β relaxation has been observed. According to some authors, it is related to the motion of hydroxyl groups connected to water molecules,[31,32] whereas other authors consider the β relaxation as a typical water relaxation.[33] In order to better classify this transition the dynamic-mechanical test was performed again in multifrequency mode. It was found that, on increasing the frequency, the peak maximum was shifted toward higher temperatures, and this allowed us to calculate the apparent activation energy of the process (E_a). The values of the activation energies, reported in Table 4, are compatible with the motion of hydroxyl groups. It is interesting to note that the T_β increases on increasing the content of guluronic moiety. This is because, on increasing the content of G-units, the stiffness of chain segments increases and the molecular mobility decreases.

On the contrary, in cross-linked samples we observe a decrease of the temperature at which the transition occurs and a decrease of the activation energy going from sample A−Ca to sample C−Ca. The more pronounced depression of the T_β, observed in the two samples with higher content of guluronic units, is a confirmation of our hypothesis. In fact from Table 2

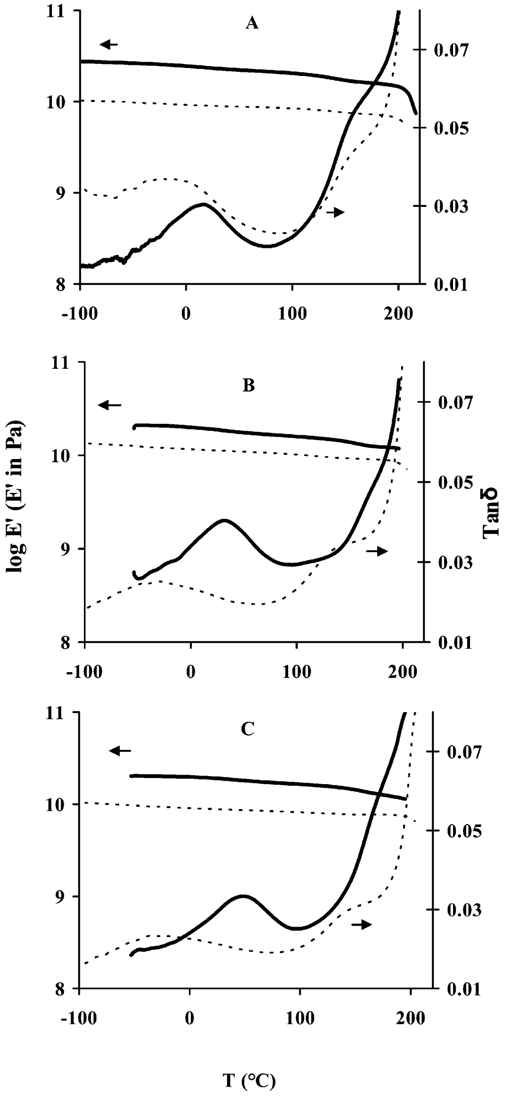

Figure 3. Storage modulus and tan δ of samples A, B, and C (—) and samples A−Ca, B−Ca, and C−Ca (− − −).

we notice that samples B and C swell much more than sample A. In addition, from Table 3, we deduce that sample B−Ca and C−Ca contain more water than sample A−Ca. Both factors, the increase of the free volume, along with the increased amount of water, contribute to lower either the temperature or the activation energy of this transition.

Permeability. The permeability data are collected in Table 4. Samples A and B have a similar permeability, whereas sample C, containing more guluronic units, has a lower value. This is reasonable because increasing the concentration of guluronic units the chain-to-chain interactions increase and act as a hindrance to the diffusing molecules making the diffusive path more tortuous.[34]

Usually when points of cross-linking are introduced in a polymeric film the permeability generally reduces. In our case the permeability of the three cross-linked samples increases. This unusual behavior is consistent with the other experimental data discussed so far. As previously stated, the samples are cross-linked in a swollen state, and the different chain segments are connected to each other when the free volume has increased. When the cross-linked samples are dried and reconditioned at room temperature and at about 50% RH, the chain segments cannot assume the conformations they had before the introduction of the tie points. As for the permeability there are two effects to be considered: the introduction of cross-linking points, that generally lowers the transport properties, and the increase of the free volume, that makes the pathway of the diffusing molecules easier with the consequent increase of the transport parameters. According to the slight increase of the permeability in the cross-linked samples, we have to conclude that the increase of free volume has more effect with respect to the introduction of cross-linking points.

Conclusions

In this work the physical behavior of three films of alginate has been studied. The three samples had similar molecular masses but different content of guluronic moieties. This was interesting because the differences in terms of physical parameters were ascribed to the different content of G. The increase of the G-units promoted the chain-to-chain interaction with a consequent increase of the T_g. The cross-linking caused a considerable change of the analyzed physical parameters in an unexpected direction. In fact, both the glass transition temperature and the β relaxation, detected with the dynamic-mechanical experiment, decreased in the cross-linked samples. This was explained in terms of increased free volume during the cross-linking process. In fact, since the introduction of the cross-linking points occurred in the swollen state, once the absorbed water was removed, the tie points introduced between different chains did not allow the chain segments to restore the conformations they had before the cross-linking. This hypothesis was supported by the swelling experiments. In fact the increase of the thickness as a consequence of the cross-linking process is proof of the increased free volume. The permeability data were supportive of this hypothesis as well.

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