Effect of Water Content on the Structural Reorganization and Elastic Properties of Biopolymer Films: A Comparative Study

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In this work, the effect of water uptake on the structural reorganization and elastic properties of three types of biopolymer films was studied. The water−biopolymer interaction for hydroxypropyl cellulose (HPC), gelatin, and cassava starch films prepared from aqueous solutions was studied and compared using Fourier-transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), X-ray diffraction, dynamic vapor sorption (DVS), and dynamic mechanical thermal analysis with humidity generator and controller (DMTA) techniques. The FTIR spectral variations due to the water sorption were generalized into two-dimensional (2D) correlation graphs for each biopolymer, and the effect of water on the molecular conformation was compared. The water sorption isotherms were fitted with Guggenheim−Anderson−De Boer (GAB) and D'Arcy and Watt models. The water content in the mono- and multilayers predicted by both models for each biopolymer was discussed and compared. The correlation of the fitted data obtained from the sorption isotherms to the DMTA data allowed us to conclude that the elastic properties of the HPC films depended on the total water content in contrast to the elastic properties of the gelatin and cassava starch films, which decrease only with the appearance of multilayer water.

Introduction

Much of the current research in material sciences is focused on the structure−properties relationship to predict and control the functional properties of the materials through their structure.[1−11] The understanding of this relationship is particularly important to allow the correct choice of material and processing method for an application, thus optimizing finished product performance.[12] The complex structure of a material can be approached as an arrangement of structural units in a particular order at different levels: from micro to macro. Therefore, to predict its macroscopic properties, the molecular organization at different structural levels should be considered.

Biopolymers are of great interest because they are made from renewable natural sources and promise a solution to the environmental problem of plastic waste disposal because of their biodegradability.[13−15] A disadvantage of biopolymers is that they naturally interact with water, which leads to water-induced textural transformations (for example, amorphous−crystalline transition) that have a strong impact on their molecular mobility and functional properties.[16−23] Consequently, the water content of biopolymer films is a critical variable in the equation properties = *f*(structure).

The primary purpose of this study was to correlate the physicochemical modifications induced by the water sorption on the elastic properties of biopolymer films. An additional objective of this study was a detailed analysis of the sorption isotherms and their modeling to highlight the differences in water−biopolymer interactions. The development of a simple mathematical model capable of predicting the elastic properties of the biopolymer films with respect to the water content was a logical final aim of this work. We have selected three biopolymers with very large structural differences that we consider representative of different types of behavior, which are gelatin, cassava starch, and HPC.

Physicochemical Characteristics of Biopolymers and Their Films. *(i) Gelatin.* Gelatin belongs to the group of animal origin protein and is well-known for its good film-forming properties.[2,3,22] It is a product of the collagen degradation, which involves the breaking of the triple-helix structure into random coils.[24] The gelatin molecular chain is a heterogeneous mixture of single or multistranded polypeptides, each with extended left-handed proline helix conformations and containing between 300 and 4000 amino acids residues.[25] The main property of gelatin is its ability to form a thermally reversible network in aqueous media. When an aqueous gelatin solution is cooled, the protein coils locally assemble into triple helical sequences connected in an essentially random mode by peptide sequences in disordered conformation forming a physical network.[26] It was previously reported that the sol→gel transition for physical aqueous gelatin gels occurs at 40 °C, and below this temperature the gel phase begins to form.[27] Subsequent drying of the physical gels leads to the formation of films with physically cross-linked and partially renaturated collagen-like structures. The mechanical and thermal properties of gelatin films formed from aqueous solutions depend on many parameters including the molecular mass and aminoacid composition, as well as environment conditions, such as the thermal history.[1,3,28] It was also previously demonstrated that the mechanical properties of gelatin films are closely related to the renaturation level.[2] On the other hand, the water content of the films plays an important role in the formation of the helical structure and consequently affects

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their renaturation level.[22,24] Therefore, the water content of gelatin films should be taken into account to predict their functional properties.

(ii) Cassava Starch. From the vast varieties of starches, cassava starch is becoming a widely used starch in the food industry because of its ability to perform most of the functions of maize, rice, and wheat starches. The cassava demand is estimated to grow at 2.0% annually for food and 1.6% per year for feed in developing countries, while the total cassava production is projected to reach 168 million tons by 2020.[15] The exceptional mechanical properties of cassava starch films were previously reported.[13,14] Mali et al. compared their properties to those of corn and yam starch films. Cassava starch showed the lowest content of amylose (19%) as compared to corn and yam starches. As amylose content affects the opacity and strength of starch films, the cassava films were found less strong, more transparent, and flexible.[5] In addition, Mali et al. demonstrated that cassava films with and without plasticizer had the highest strain at break and lowest tensile stress and Young modulus as compared to those of corn and yam starches.[6]

As with all starches, cassava consists of two polysaccharides. Amylose has long linear chains of (1→4)-linked α-D-glucopyranose residues, some with a few branches. Amylopectin has high molecular weight and highly branched structure consisting of much shorter chains of (1→4)-linked α-D-glucopyranose residues.[29] The amylose content changes with the botanical source from 20% to 30% and greatly affects the functional properties of the derivative products from starches. It was previously shown that the mechanical properties of plasticized starch films depend on the amylose/amylopectin ratio of starch, because the linear amylose and the branched amylopectin exhibit a different behavior with regard to the gelation process and film forming capacity.[30] During the heating process, the starch granules start to swell, rupture, and collapse, releasing the amylose and amylopectin. The branched amylopectin chains in solution have a little tendency to interact, and, consequently, amylopectin gels and films are weak, cohesive, and flexible. In contrast, the linear chains of amylose in solution have a higher tendency to interact via hydrogen bonds. Thus, amylose gels and films are stiffer and stronger than amylopectin gels and films.[7] After gelatinization, the starch paste contains a mixture of the dispersed amylose and amylopectin, which leached out from the granules and might also include "ghosts" (remnants of the granular envelope).[31−33] Under certain conditions, the starch films could form amylose-rich and amylopectin-rich phases, which results in a phase-separated structure. The latter introduces the heterogeneity of structure and also affects the properties of the starch films.[34−36]

(iii) Hydroxypropyl Cellulose. HPC is a chemically modified cellulose manufactured by reacting alkali cellulose with propylene oxide, which makes it water-soluble. This chemical reaction leads to the propylene oxide substitution on the cellulose through an ether linkage at three reactive hydroxyls present on each anhydroglucose monomer unit of the cellulose chain. Therefore, the degree of substitution (DS) and the molar substitution (MS) of the HPC are important characteristics related to its physicochemical properties. Another feature particularity of the HPC is its ability to form cholesteric liquid crystalline order in water. Wirick and Werbowyj first reported that HPC forms a liquid crystal structure in concentrated water solution.[37,38] To conserve these cholesteric ordered structures in the HPC films, different cross-linking methods can be used, such as γ-ray irradiation.[39] However, it was reported that HPC films prepared from solutions based on organic solvents without employing any cross-linking method still contained the ordered phase due to the residual liquid crystal superstructure.[40] It was also shown that different methods of preparation of HPC films resulted in different structural order, which has a significant impact on their mechanical properties and could even lead to their anisotropy.[41]

Water Sorption: Monolayer and Multilayer. The hydrophilic nature of biopolymers is the main limitation for the application of biodegradable materials. A common approach to describe water sorption processes is by using models based on the assumption of a fixed number of binding sites, to which the water molecules first attach forming a monolayer, followed by multilayers of much more loosely held water. Additionally, the monolayer could be divided into primary strong adsorption to the hydrophilic sites and weak primary sorption to the hydrophobic sites.[42−44] Hence, the analysis of an adequate sorption isotherm model can provide important information regarding the water binding on the biopolymers. **Viscoelastic and Fracture Properties of Films.** The main mechanical characteristics of biopolymer films can be determined from the stress−strain diagram obtained from tensile tests. In addition, the viscoelastic properties of polymeric materials can be elucidated through the evaluation of the storage modulus E' and loss modulus E'' as a function of both frequency and temperature. Numerous authors reported the plasticizing effect of water on the mechanical properties of the biopolymer films, manifested by a decrease in Young's modulus (or elasticity modulus) and stress strength.[17,19,20,45] Zhou et al. studied the plasticizing effect of water on the structure and properties of regenerated cellulose by cross-correlation analysis of the results obtained from simultaneous measurements carried out with different techniques. It was shown that the storage modulus measured by DMTA exhibited a deflection point at 40% and 80% relative humidity (RH) related to changes in the dynamic mechanical behavior of the regenerated cellulose films. The correspondence of the transitions in the mechanical behavior to those in the infrared spectra measurements indicated that Young's modulus was affected by the increase of sorbed water molecules.[46] In addition, Mali et al. demonstrated that the elasticity modulus and the tensile stress of cassava starch films were strongly affected by the water content when a critical value of 58% RH was reached, even when plasticizers such as glycerol and sorbitol were employed.[6] These findings suggest that the properties of biopolymer films are closely related to their sorption behavior.

Materials and Methods

Film Preparation and Conditioning. The gelatin used in this study was B type from bovine skin (Sigma-Aldrich Ltd., Poole, UK, G9382), bloom 225 with a molecular weight (MW) of 142 000 g/mol determined by size exclusion chromatography as described by Wulansari et al.[47] Hydroxypropyl cellulose was from Klucel, Hercules Inc., LF type with a molar substitution of 3.8 and a molecular weight of 95 000 g/mol according to the manufacture specification. Native cassava starch (National Starch and Chemical Corp., Springfield, NJ) contained 18.0 ± 0.9% of amylose content as determined by the DSC method described by Mestres et al.[48]

Aqueous solutions (4:100 w/w) were prepared from gelatin (70 °C, 30 min), HPC (22 °C, 5 h), and cassava (90 °C, 1 h, water bath) powders. Subsequently, the solutions were cast in polystyrene Petri dishes and dried in controlled conditions (20 °C and 45−55% RH). To perform the hydration by sorption, the films were predried (40 °C, oven) and subsequently equilibrated in different relative humidities at 25 °C for at least 1 week. Six salt solutions, LiCl (11.3 RH), CH_3-COOK (21.6 RH), $MgCl_2$ (32.8 RH), K_2CO_3 (44 RH), NaBr (57.5 RH),

KI (68.9 RH), and P_2O_5 powder (~0 RH) were used to maintain the specified relative humidities in sealed chambers.

For tensile tests, films with a thickness of approximately 100 μm were cut into strip specimens with 10 mm width and 100 mm length. For DSC and X-ray analysis, the films were milled in a mortar with liquid nitrogen to avoid heating effects. For FTIR analysis, thin films (~15 μm) were prepared by casting the solutions into 5 cm plastic Petri dishes using a spin-coater (SCS-G3P8, SCS Inc., Indianapolis, IN) running at 300 rpm for 25 s. The films were left to dry on a leveled surface and then cut into 5 mm × 30 mm strips.

Fourier-Transform Infrared Spectroscopy. The film strips prepared for FTIR analysis were attached to metal holders in a hydration cell with BaF_2 windows. The humidity inside the cell was controlled by mixing a stream of dry (0.0% RH) and wet (100% RH) air. The cell was mounted under an UMA500 FTIR microscope interfaced to a BioRad FTS175C Fourier-transform spectrometer. A background spectrum of the cell without film was recorded, and then the IR beam was focused on the film and the sample spectrum was collected. 256 scans at 2 cm^{-1} resolution were averaged. Water vapor bands were subtracted manually using the spectrometer software (WinIR Pro, BioRad). Further analysis was done using MATLAB (MathWorks, Inc., Natick, MA). Difference spectra were calculated by subtracting the spectrum of the dry film (0% RH) from the spectra of sequentially hydrated films with a constant subtraction factor of 1.0. The spectra were baseline-corrected by removing linear offsets (absorption at 1800 cm^{-1} set to 0), and then the difference spectra were arranged in a matrix with increasing hydration. Statistical 2D correlations were calculated from the covariance and correlation matrices using a variation of the method described by Šašić and Ozaki.[49]

X-ray Diffraction. X-ray diffractograms were recorded on films for 2θ between 4 and 38 at 0.1° intervals (scanning rate 6 s^{-1}) using a Bruker D5005 (Bruker AXS, UK) diffractometer equipped with a copper tube operating at 40 kV and 40 mA producing Cu Ka radiation of 1.54 Å wavelength.

Optical Microscopy. To compare the microstructure of the biopolymer films, light micrographs were collected using a Leitz Diaplan (Germany) microscope with a Pixelink PL-A600 camera.

Dynamic Vapor Sorption and Data Fitting. Dynamic vapor sorption analyzer DVS-1 (Surface Measurement Systems Ltd., London, UK) equipped with a Cahn D200 microbalance was used to measure the water sorption isotherms of the biopolymer films. The experiments were carried out at ambient temperature (25 °C) and relative humidity ranging from 0% to 90% RH. The initial weight of the samples was approximately ~7 mg. The samples were predried in the DVS-1 by exposure to dry air until the dry weight of the samples was equilibrated. The dry samples were subsequently hydrated stepwise in 10% RH steps, by equilibrating the sample weight at each step. The samples were considered equilibrated when the slope $\Delta m/\Delta t = 0.0005$ mg/min or equilibration time exceeded 700 min.

To compare the sorption behavior of the biopolymer films, the experimental data were fitted. Two models were chosen: the Guggenheim−Anderson−De Boer (GAB) equation for its wide use for food materials and D'Arcy and Watt for its simplicity to describe three stages of sorption.

The GAB model is mathematically expressed as:

$$M = \frac{M_0 CKa_w}{(1 - Ka_w)(1 - Ka_w + CKa_w)} \quad (1)$$

where M is the equilibrium moisture content (g water/100 g dry solids), M_0 is the water content in the monolayer (g water/100 g dry solids), a_w is the water activity, C is a constant related to the monolayer enthalpy of sorption, and K is a constant related to the multilayer heat of sorption. The constants C and K are temperature dependent.[21,50] The GAB model can be split into multilayer and monolayer water content according to the following equations:[18]

$$M_{mon} = M(1 - Ka_w) \quad (2)$$

$$M_{mult} = MKa_w \quad (3)$$

where M_{mon} and M_{mult} are the equilibrium moisture content in the monolayer and multiplayer, respectively (g water/100 g dry solids).

The D'Arcy and Watt model was developed to describe the water sorption in heterogeneous materials and is composed of three terms, each representing a different type of water binding in the material.[51] The first term (monolayer-region 1) describes binding sites where individual water molecules bind strongly (Langmuir sorption), the second (monolayer-region 2) describes sites where clusters of water bind weakly (Henry sorption), and the third (multilayer-region 3) describes sites where water arranges as a collection of molecules. To decrease the number of adjustable parameters, the modified D'Arcy and Watt model was used in this study:[52]

$$M = \frac{KK'a_w}{1 + K'a_w} + ca_w + \frac{ba_w}{1 - ba_w} \quad (4)$$

where a_w is the water activity, K and K' are the affinity and number of strong binding sites, respectively, c is a function of the number and strength of weak binding sites, and b is a function of the number and strength of multi-molecular sorption sites.

The goodness of fit for both models was evaluated using the mean relative deviation modulus (P), defined by:

$$P = \frac{100}{n} \sum \frac{|M_i - M_{Ei}|}{M_i} \quad (5)$$

where M_i is the experimental moisture content at experiment i, M_{Ei} is the predicted moisture content, and n is the number of experiments.[53,54]

Differential Scanning Calorimetry. A Perkin-Elmer DSC 7 (Norwalk, CT) differential scanning calorimeter was used in this study. The instrument was calibrated with pure indium and cyclohexane. Samples (~18 mg) with different water contents were sealed in stainless steel pans and heated at 10 °C/min. An empty pan of the same type was used as reference. The measurements were performed in the temperature range between −20 and 200 °C. After the first heating, the samples were rapidly cooled (50 °C/min) and then reheated at the same rate of 10 °C/min. Two thermal characteristics were measured: (1) enthalpy of melting (ΔH_m) in the first heating scan (only for gelatin films) and melting temperature reported as the peak of the melting peak; and (2) glass transition temperature (T_g), reported as the midpoint of the ΔC_p changes at the glass transition measured in the second heating scan.

Mechanical Tests. Tensile tests were conducted on a Texture Analyzer (TA) TA.XT plus (Stable Micro Systems, Surrey, UK) with a 30 kg load cell, using the tensile grips (A/TG). The tests were performed at ambient temperature at a speed of 10 mm/min with 50 mm gauge length. The low-speed regime was chosen due to the brittleness of the biopolymer films with low water content. The hydrated tensile specimens (from 0% to 75% RH with saturated salt solutions) were coated in silicon oil to avoid water loss during the tensile test. To evaluate the elasticity of the films with respect to water content, at least 10 specimens (thickness = 0.1 ± 0.08 mm, width = 10 mm, and length = 100 mm) for each hydration level were stretched. The recorded tensile test data, force = f(distance), were converted into the conventional stress−strain ($\sigma_{con} = f(\epsilon_{con})$) diagrams using the dimensions of each sample.

Dynamic Mechanical Thermal Analysis. To measure the dynamic mechanical characteristics of the biopolymer films, a Triton Tritec DMTA equipped with a humidity generator and controller (Triton Technology Ltd., Keyworth, UK) was used. Films with 5 mm width, 25 mm length, and 100 μm thickness were used. A sinusoidal load, automatically adjusted to achieve a displacement of 0.010 mm at the frequencies 1 and 10 Hz, was applied in tension mode with a gauge length of 5 mm. The measurements were carried out at 23 °C with

relative humidity increasing linearly from 30% to 80% at 1%/min. In the present work, the water content of the samples under hydration ramp conditions was estimated using the model parameters obtained from the fit of the DVS sorption isotherms. The disadvantage of this technique is the difficulty to calculate the changes in the weight of the samples at a given relative humidity, and consequently it is not possible to calculate the real water content of the samples during the experiments. Moreover, the humidity ramp rate does not allow the samples to reach the water content at equilibrium. Therefore, the measurement of the storage modulus of the biopolymer films via DMTA under dynamic hydration conditions was employed only as a complementary technique to compare to the elasticity modulus obtained from tensile tests (TA).

Results and Discussion

2D FTIR Correlation Spectral Changes with Hydration for the Three Biopolymers. Infrared spectroscopy is a valuable tool to study biopolymer structures. However, the analysis of hydration effects is hampered by the strong stretching and deformation bands from the adsorbed water molecules. Reliable subtraction of the water contribution is difficult, because the IR absorptions of bound water molecules may be different from those of free water. 2D analysis has been used successfully to disentangle spectra of mixed systems and could even resolve strongly overlapping peaks.[55,56]

The statistical two-dimensional FTIR correlation spectra technique described by Šašić and Ozaki was used to evaluate the hydration effect on the conformational changes and deformation of the molecular bands in the gelatin, cassava starch, and HPC films.[49]

The hydration behavior of gelatin films has been discussed in detail in our previous work.[22] It was found that water sorption caused two main peaks in the difference spectra at 3475 and 3240 cm^{-1}, due to the ν_{as} and ν_s OH stretching bands. In the amide I region, a negative band at around 1695 cm^{-1} (due to the loss of aggregated helices) and a positive band at 1630 cm^{-1} (due to the formation of hydrated chains of predominantly coil structure gelatin) were overlapped with the deformation band of adsorbed water molecules. In the amide II region, a positive band at 1560 cm^{-1} and a negative band at 1500 cm^{-1} indicated increasing levels of the protein backbone hydration.

Figure 1a shows the synchronous 2D FTIR correlation maps for hydrating gelatin films, plotted in the range from 800 to 3800 cm^{-1}. Cross-peaks are shown on the synchronous plot when bands change simultaneously. The two types of lines were used to indicate the direction of the change: solid lines show bands that move in the same direction, and dotted lines represent the bands that move in opposite direction. (Diagonal peaks always correlate positively with themselves.) There were clear cross-peaks between all of the difference bands in the amide region, consisting of two pairs, 1695−1510 and 1620−1575 cm^{-1}. The correlation peaks were also observed with CC bands at ~1440 cm^{-1} and with the amide III band at ~1220 cm^{-1}. It is also shown in Figure 1a that two OH bands at 3475 and 3240 cm^{-1} correlated strongly with themselves as well as with all of the amide bands. The asynchronous correlation plot (Figure 1b) was noisy, and only weak peaks were seen, predominantly from the background, and no peaks were observed in the OH region. However, there were small cross-peaks between amide I band components at 1660, 1643, and 1633 cm^{-1}, which have been assigned to triple helical "collagen" structure, unordered coil structure, and β-sheet. These reflect the protein structures and interactions at low and intermediate water contents, which have been reported previously.[22]

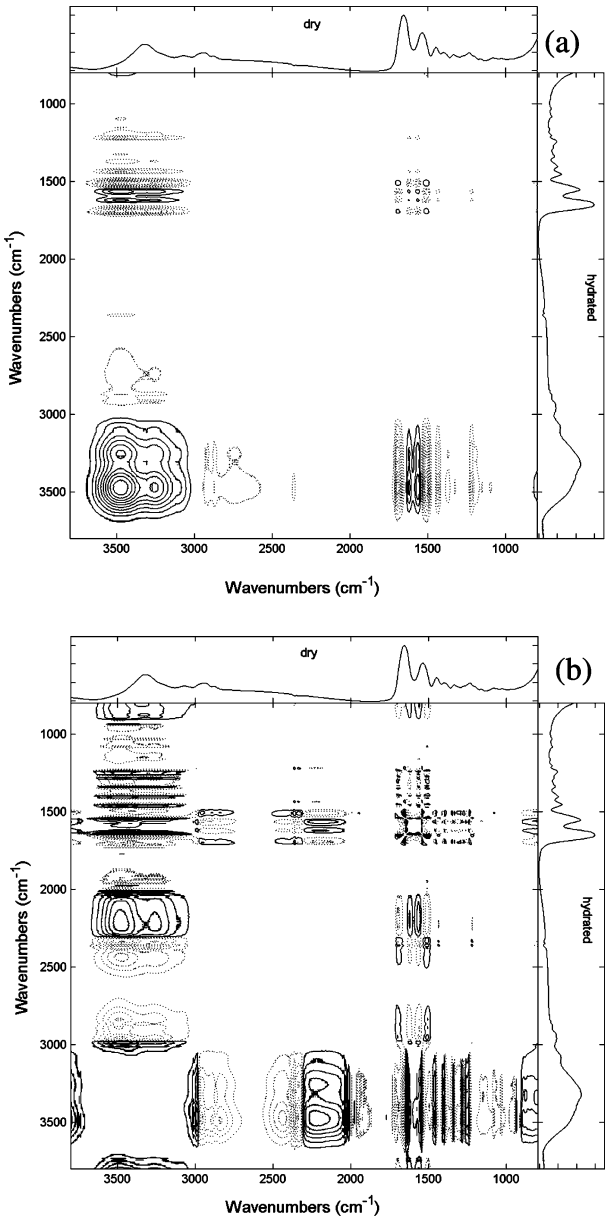

Figure 1. 2D FTIR correlation maps in the range of 800−3800 cm^{-1} for the hydrating gelatin films: (a) synchronous and (b) asynchronous. The spectra of dry and fully hydrated gelatin are plotted alongside to show the spectral changes.

Thus, the synchronous and asynchronous FTIR correlation maps for gelatin films indicated a very strong correlation of the protein backbone hydration with the water uptake, but only limited changes in secondary structure.

For HPC films, the synchronous plot (Figure 2a) showed a strong correlation between the water bands (~3550 and 3300 cm^{-1}, 1630 cm^{-1}) and carbohydrate bands around 1060 cm^{-1}. There were additional cross-peaks in the 1200−1500 cm^{-1} region. The decrease of the 1376 cm^{-1} peak, accompanied by two positive side lobes, indicated that this feature resulted from band broadening. In the asynchronous correlation plot (Figure 2b), there was only one significant set of cross-peaks between the OH bands and HPC bands at 1080 and 1125 cm^{-1}, both of which have been assigned to C−O−C modes from side chains.[57]

Because the other carbohydrate bands were correlated with the OH bands, this was interpreted as a continuous hydration process without any sudden structure changes in the HPC polymer, but some nonlinear effect on the intermolecular interaction of HPC molecules.

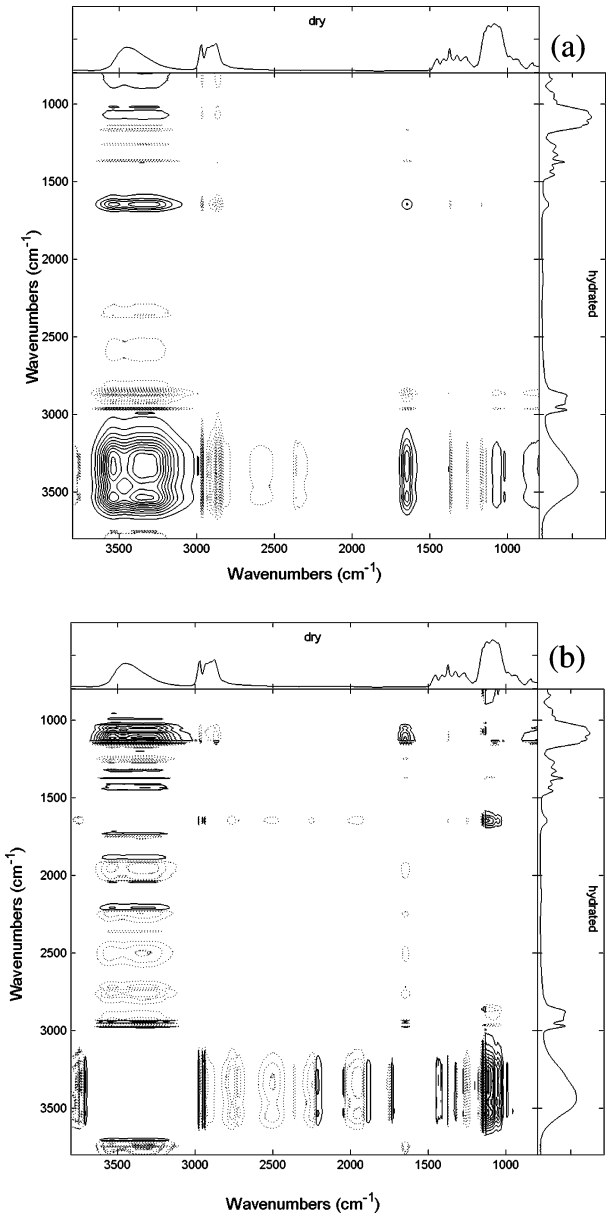

Figure 2. 2D FTIR correlation maps in the range of 800−3800 cm^{-1} for the hydrating HPC films: (a) synchronous and (b) asynchronous. The spectra of dry and fully hydrated HPC are plotted alongside to show the spectral changes.

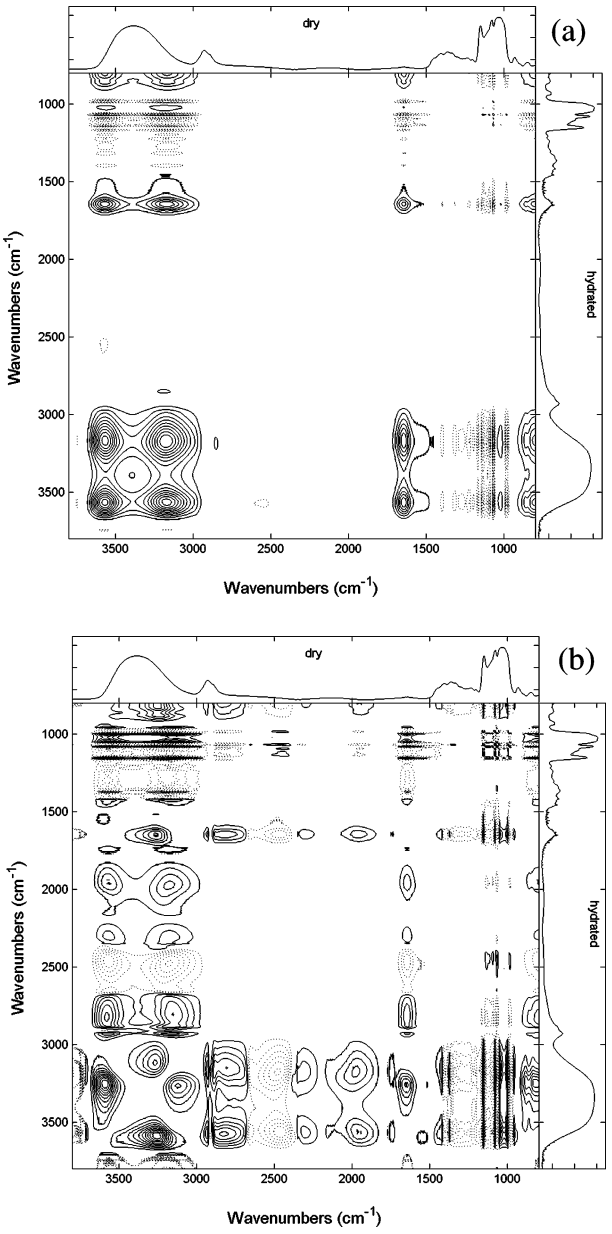

Figure 3. 2D FTIR correlation maps in the range of 800−3800 cm^{-1} for the hydrating cassava starch films: (a) synchronous and (b) asynchronous. The spectra of dry and fully hydrated cassava starch are plotted alongside to show the spectral changes.

The cross-peaks between water and carbohydrate bands in the synchronous correlation map for cassava starch films (Figure 3a) demonstrated changes linked to the water uptake. However, the major difference between the previously discussed gelatin and HPC systems and cassava was the appearance of strong OH cross-peaks also in the asynchronous correlation map (Figure 3b). The observed cross-peaks between the 3247 cm^{-1} peak and the peaks at 3580 and 3187 cm^{-1} indicated major changes in the adsorbed water. The band at 3580 cm^{-1} due to "free" water was small at low hydration but increased strongly as compared to the "bound", that is, hydrogen-bonded water molecules. Moreover, the band found at 3247 cm^{-1} in the first steps of hydration shifted to 3187 cm^{-1} in higher hydrated films. The 3247 cm^{-1} peak did not correlate with the OH deformation band either. While the cross-peaks in the synchronous map indicated that the major hydration changes in the carbohydrate region were correlated with the 3187 and 3580 cm^{-1} peaks, the asynchronous map also showed cross-peaks in the 1000−1200 cm^{-1} region, which were not. Together with the 3247 cm^{-1} peak observed at low water content, this could be interpreted as a structural change in the starch linked to strongly bound water molecules at low water content, whereas more loosely bound (free) water did not affect the structure in the same way. Also, because no changes were observed in bands at 1020 and 1047 cm^{-1}, there appeared to be no effect of hydration on the level of crystallinity in the cassava starch films.

Effect of Water on the Structure of Biopolymer Films. To demonstrate the structural differences and the water effect on structural reorganization of films, light microscopy and physicochemical analysis by X-ray diffraction and DSC were performed (Figure 4).

The gelatin films were optically clear, and no structural order could be observed. It was also reported previously that the surface of collagen-based films is homogeneous and smooth and no structure was visible even at high magnifications.[58] On the molecular level, the X-ray patterns of gelatin films showed the presence of a small amount (from 2% to 11%) of triple-

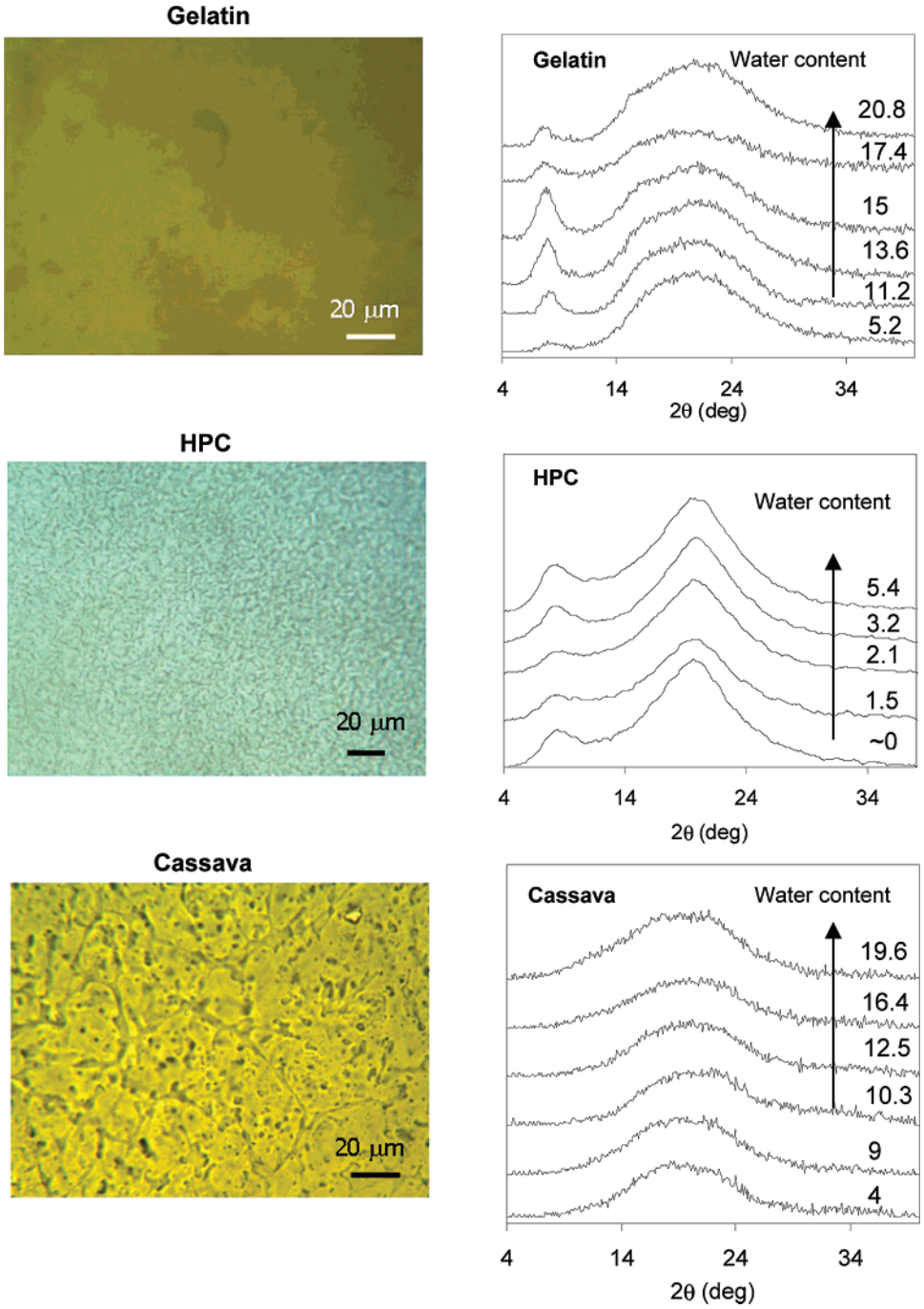

Figure 4. Optical micrographs of biopolymer films at room conditions (T = 22 °C, RH = 50−60%) and X-ray patterns of biopolymer films hydrated at different levels (water content on dry basis).

helical structure ($2\theta = 8°$) corresponding to the periodicity from 8.6 to 14.5 Å, characteristic of collagen rod-like triple helices, 300 nm long and 1.5 nm wide, and the absence of any particular orientation in coil-like amorphous phase. In addition, the proportion between the coil and triple-helix structure was changed with an increase in the water content of films. X-ray patterns indicated that the maximum relative content of triple-helical structure (9−11%) corresponded to the medium range of water content, which could be assigned to the sorption of water molecules by hydrophobic sites located inside of the triple-helices.[22,24]

Light microscopy of HPC films indicated the presence of a residual microstructure (~1 μm), which could be related to a particular organization of the HPC molecules into rich and non-rich regions during the film formation.[59] It is also well known that HPC forms a cholesteric liquid crystal order or mesophase in aqueous solution with a d spacing ranging from 12 to ~13.5 Å, which increases with decreasing mesophase concentration.[60,61] It was also previously demonstrated that in the aqueous solution the cellulose chains are stiffened by van der Waals forces and by inter- and intramolecular hydrogen bonding and form microfibrils.[4] Moreover, it is well known that the hydroxypropyl groups are rather hydrophobic, which results in lower affinity of HPC for water and gives an adequate condition for the formation of an ordered phase. In our work, the X-ray patterns of the HPC films showed the presence of some preserved ordered structure, which is manifested by a broad peak at $2\theta = 8.4°$. These results are in agreement with those reported by Samuels who studied the properties of the water-cast and

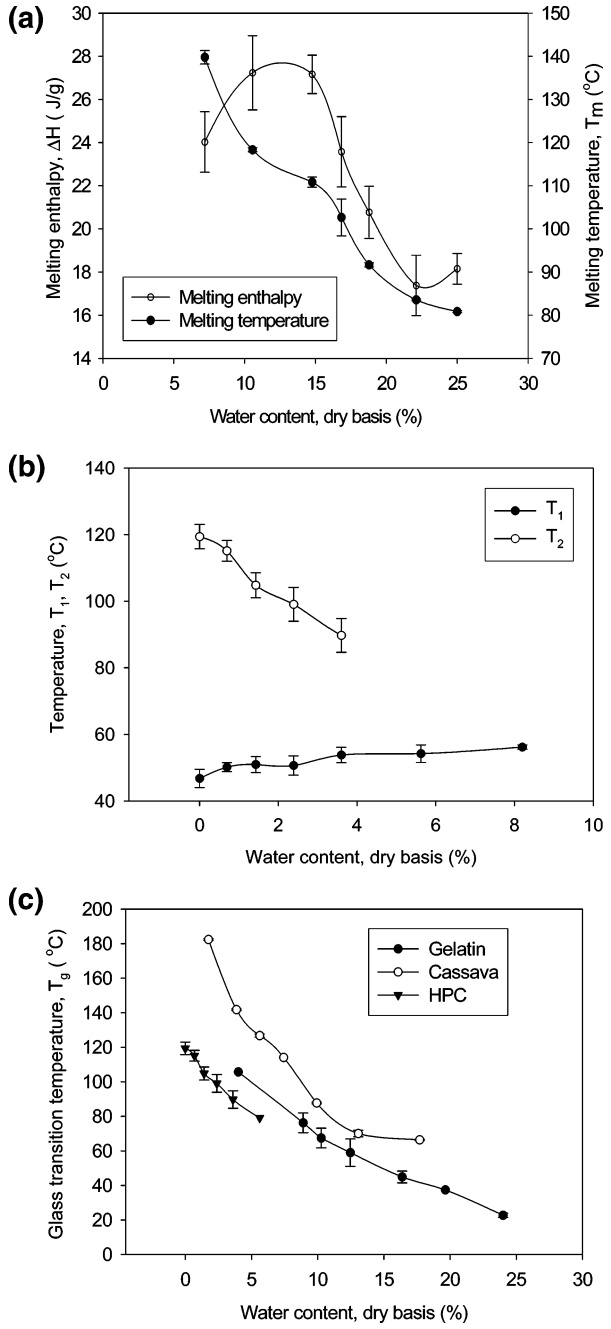

Figure 5. Effect of water on the melting temperature and enthalpy in gelatin films (a); two transitions in HPC films (b); plasticizing effect of water on the glass transition for three biopolymers (c).

oriented HPC films and demonstrated that the polymer crystallized as an irregular three-fold helix.[57]

Evmenenko et al. suggested that the structural behavior of cellulose derivative films such as HPC must be related to the substituents and the conformation changes (steric effects) at high polymer concentrations during the film formation. The ability to form the mesophase was also related to the degree of the macromolecules anisodiametry. Formation of intra- and intermolecular hydrogen bonds led to the appearance of rigid extended mesogenic fragments and increased anisodiametry.[4] In our work, the X-ray diffraction of the HPC films also revealed a broad peak at $2\theta = 20°$ (this peak comprised the d spacing from 3 to 6 Å), which confirmed that the structure of the films was predominantly composed by molecules orientated to a certain extent. It is rather difficult to depict the effect of water content on the structural reorganization of the HPC films from

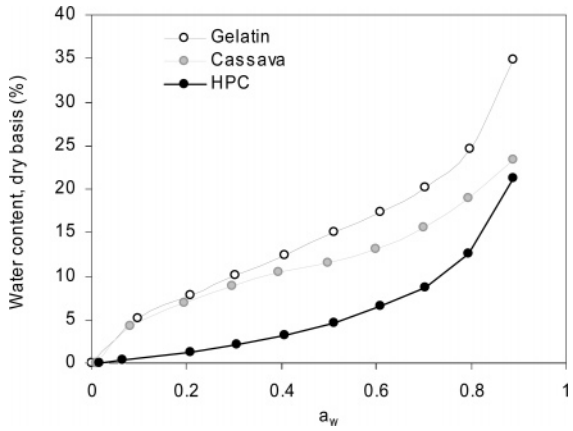

Figure 6. Sorption isotherms for gelatin, cassava, and HPC films obtained by DVS.

the X-ray patterns. However, it was clearly shown that the water content did not greatly affect the structural order of the HPC films.

Cassava films showed a completely different structural organization. The micrographs clearly showed the presence of the granule remnants ("ghosts") that are primarily composed of amylopectin and exhibit elastic/plastic properties that could influence the properties of the films.[62] (According to Atkin et al., "ghosts" are defined as the remnants of the envelope after structural collapse where the majority of the starch polymers have been released.[63]) In addition, no phase separation was observed probably due to the presence of the ghosts, which allowed the maintenance of the network of amylose/amylopectin. On the other hand, the X-ray patterns revealed that this complex structure was completely amorphous even at high water content. This fact shows that the amylose, which is mainly responsible for the crystallinity of the starch films, under the described film-forming conditions, did not organize in an ordered structure by forming a crystalline phase. Moreover, this amorphous structure seemed to be stable, and even at high water content the sorbed water molecules did not promote the molecules reorganization.

The DSC results shown in Figure 5 were consistent with the X-ray analysis and microscopy observations discussed above. In the case of gelatin films (Figure 5a), the melting peak indicated the presence of the triple-helical structure. The maximum melting enthalpy, corresponding to the maximum ordered structure, was found in the middle range of water content (7−16% dry basis), which was assigned to the water absorbed inside helices. For HPC films (Figure 5b), two relaxations were found: one at approximately 50 °C (T_1) and one in the range of 90−120 °C (T_2). It was previously reported that the dynamic mechanical properties of HPC films change only at a higher temperature transition (T_2).[63] Consequently, the first transition at the lower temperature (T_1) probably occurred due to the movement of the hydroxypropyl substituents, and the second transition at higher temperature (T_2) could be assigned to the glass transition.[63] These results are in good agreement with the transition temperatures found in the literature.[60,63,64] The obtained results suggested that the glass transition of HPC films was affected by the water content, as expected for a biopolymer, while the transition allocated to the movement of substituents was not affected by the water content. For cassava starch films, the DSC patterns did not show the melting point, which confirmed the amorphous structure of the films shown by the X-ray diffraction patterns.

The comparison of the glass transition for the three biopolymers (Figure 5c) demonstrated the well-known plasticizing effect

Table 1. Parameters from the GAB and D'Arcy and Watt Models and the Mean Relative Deviation Modulus (*P*) for Three Biopolymer Systems

	GAB				D'Arcy and Watt				
	M_0	*c*	*K*	*P*	*K*	*K′*	*c*	*b*	*P*
HPC	4.82	1.34	0.87	1.03	4.70	~0	7.96	1.02	14.61
cassava	8.99	4.07	0.69	4.51	3.26	2.07	14.11	0.95	3.67
gelatin	10.15	5.17	0.78	1.92	1.85	4.84	22.23	1.02	2.11

of water, which results in a decrease of the glass transition temperature (T_g) with increasing water content. Moreover, the detailed study of the microstructure and physicochemical characteristics of the biopolymer films highlighted their structural particularities and their different sensibility to the structural reorganizations due to the changes in the water content. These results demonstrated that to perform a comparative study the consideration of the structural organization of the biopolymer films and the effect of the water content at different structural level is essential.

Water Sorption Isotherms. The difference in the water sorption behavior of the studied biopolymer films was evaluated by DVS analysis. The sorption isotherms (Figure 6) clearly demonstrated that for the whole range of water content the gelatin films were more hydrophilic than cassava films, which in turn were more hydrophilic than HPC films.

To compare the water interaction with the biopolymers at different stages of sorption, the experimental data were fitted with two classical models: GAB and D'Arcy and Watt. The fitting parameters for both models are presented in Table 1.

The fitting results demonstrated that:

(1) The GAB model gave a good overall fit for the sorption isotherms of all biopolymers, in particular for the HPC films ($P = 1.029$).

(2) According to the mean relative deviation modulus *P*, the D'Arcy and Watt model provided an unsatisfactory fitting for the HPC film sorption isotherm ($P = 14.61$). On the other hand, the values predicted by the D'Arcy and Watt model for cassava and gelatin films were in good agreement with the experimental values.

The GAB model is based on the assumption that (1) the water molecules are sorbed into primary sites and subsequently into multilayers and (2) all multilayer states are identical.[18] On the other hand, the D'Arcy and Watt model was developed on the hypothesis that three distinct types of sorption water (strongly and weakly bound monolayer sorbed water and multilayer sorbed water) exist.[51] The sorption isotherms of gelatin and cassava films showed a fast adsorption of the water molecules at low relative humidities. This fact indicated the presence of strongly bound water and suggested that the three types of sorption, strongly and weakly sorbed monolayers and the multilayer, are well defined for these two biopolymers. Consequently, the D'Arcy and Watt model gave a more appropriate fitting for the cassava and gelatin films than the GAB model, which does not consider three types of sorption. In contrast, the HPC films did not exhibit a high adsorption activity at low relative humidities, and according to the D'Arcy and Watt model the amount of water molecules bound by hydrophilic sites is close to 0 ($K' \approx 0$), which could be explained by the high degree of substitution of this cellulose derivative. Therefore, the D'Arcy and Watt model was not suitable to predict the sorption behavior of the HPC films.

To demonstrate the differences between the two models, the monolayer and multilayer water content was estimated with both models (Figure 7). For the D'Arcy and Watt model, it was assumed that the monolayer water content is equal to the sum of Langmuir and Henry layers (first and second terms of the model). It was shown that the amount of multilayer water predicted by the GAB model with eqs 2 and 3 became important at 20% RH, whereas according to the D'Arcy and Watt model the multilayer appeared around 50% RH. It was reported previously by numerous authors that the appearance of the water packed into multilayers for gelatin, HPC, and the D'Arcy and Watt model could more accurately predict the sorption behavior of these biopolymer films.[6,22,24,51]

In conclusion, the results showed that the D'Arcy and Watt model is not mathematically "flexible" enough to give a good fit for the sorption isotherm of a biopolymer such as HPC, which does not exhibit a well-defined three sorption stages. However, even for this type of material, the D'Arcy and Watt model predicted a very reasonable amount of water molecules arranged in the mono- and multilayer. On the other hand, this model gives an accurate fit for more hydrophilic biomaterials such as cassava and gelatin films as well as the amount of water sorbed into mono- and multilayer. The GAB model demonstrated a great capability to predict the total water sorption for the three biopolymers, and in particular for the HPC films (demonstrated by low relative deviation modulus), which justifies the wide use of this model for food products. However, the amount of water in the mono- and multilayers predicted by GAB model according to eqs 2 and 3 had less physical meaning than those predicted by the D'Arcy and Watt model in particular for gelatin and cassava films. The present study demonstrated that the choice of the model to fit the sorption isotherm of biopolymers should be based on the knowledge of its interaction with water molecules and the single use of a statistical deviation parameter was not sufficient to evaluate the adequacy of the model.

Effect of the Water on Elastic Properties of the Biopolymer Films. The effect of the water content on the elastic properties of the biopolymer films was estimated by the evaluation of the elasticity modulus with respect to the water content. Two techniques were employed: the evaluation of the elasticity modulus from the stress−strain diagram obtained from the tensile tests performed on the biopolymer films statically equilibrated at different relative humidities (using saturated salt solutions chambers) and the measurement of the storage modulus by DMTA under dynamic hydration (linear increased hydration by a controlled humidity air in a humidity chamber). The results are illustrated in Figure 8.

The results showed that in the dry state the gelatin films had the highest elasticity modulus as compared to the cassava and HPC films with both techniques. It was also shown that independently of the mode of hydration (static or dynamic) and technique employed for the modulus measurements the elasticity of the HPC films strongly depended on the water content and even a small increase in the water content led to a relatively large decrease in the elasticity modulus. In contrast, the DMTA measurements of the storage modulus showed that the gelatin and cassava starch films' elastic properties started to be affected only when the water content reached a critical value. The same effect could be also observed from the measurements of the elasticity modulus calculated from the elastic part of the stress−

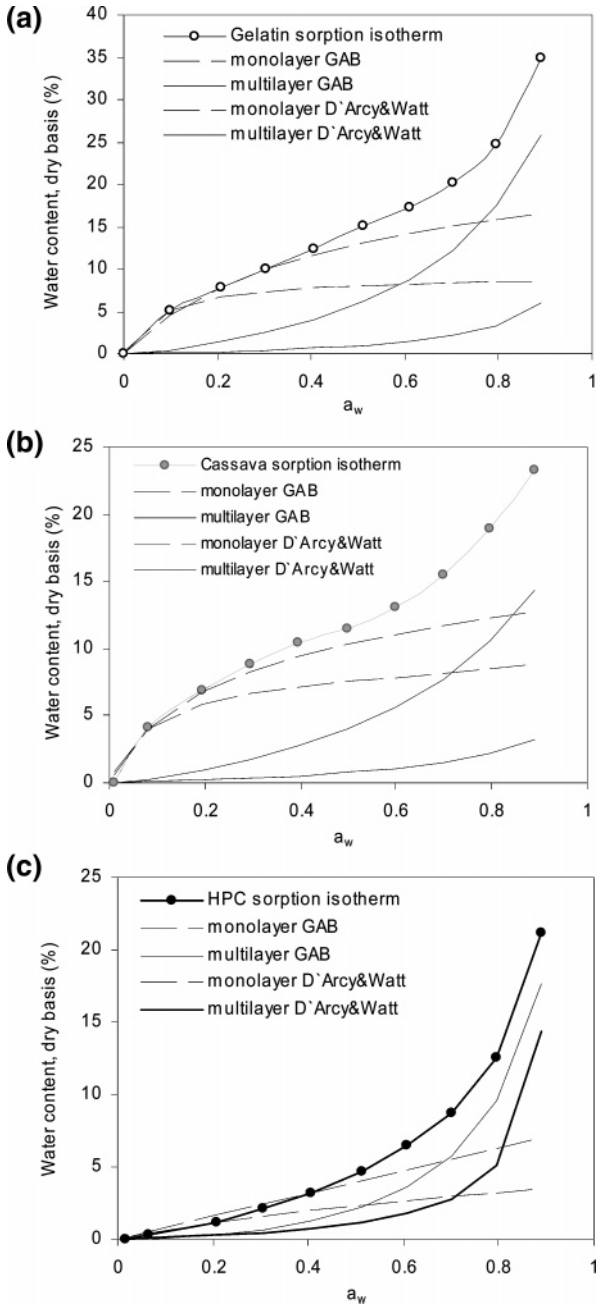

Figure 7. Water content in the monolayer and multilayers according to the GAB and D'Arcy and Watt models for gelatin (a), cassava starch (b), and HPC (c) films.

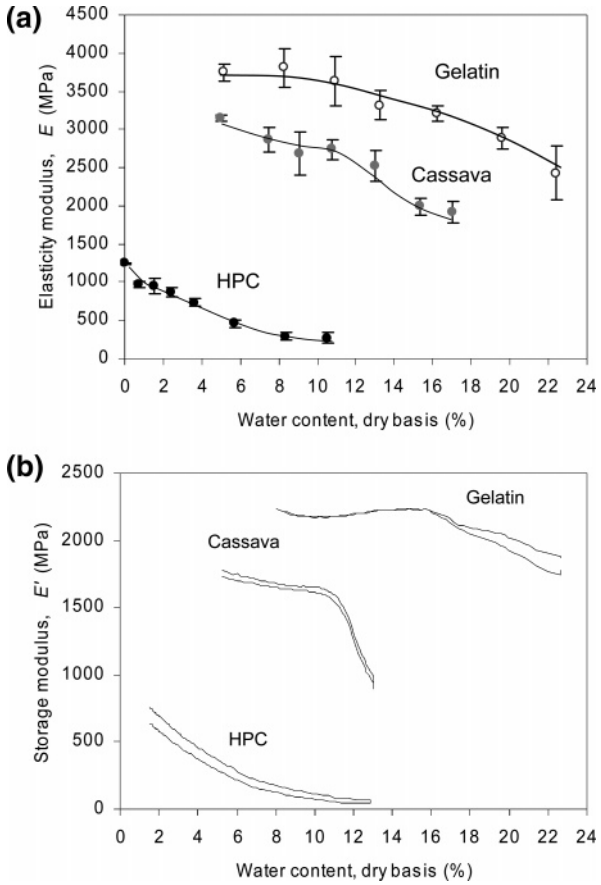

Figure 8. Effect of water content on (a) the elasticity modulus calculated from the $\sigma = f(\epsilon)$ curve obtained by TA and (b) the storage modulus obtained by DMTA at 1 Hz (gray) and 10 Hz (black) for HPC, gelatin, and cassava starch films.

strain tension diagram (tensile test). The high sensitivity of the DMTA to the changes in the storage modulus was used in this study to confirm the trend of the "elasticity modulus" = *f*(water content) curve (Figure 8a) obtained via TA. The agreement of the results suggested that the DMTA analysis under dynamic hydration conditions could be used as a complementary technique, although the equilibrium state is not reached. Figure 8 shows that these critical values for cassava starch and gelatin films corresponded to 10% and 16% of water content, respectively. These results were in agreement with the results reported by Mali et al., who identified a significant decrease in the elastic properties of cassava starch films at 58% RH.[6] Mali et al. demonstrated that the Young modulus and the tensile stress of the cassava films were strongly affected by the water content above 58% RH, which corresponded to 10% water content on dry basis. This fact was not explained, and a more detailed study of the isotherm sorption behavior was suggested to understand this properties transition.[6] The sorption isotherm fit using the D'Arcy and Watt model for cassava and gelatin films indicated that the water molecules sorbed above these concentrations get organized into multilayers because of the saturation of the vacant sites offered by biopolymer to bind the molecules of water (Figure 7a and b).

Therefore, it was concluded that the decrease of the elastic properties of cassava and gelatin films was related to the appearance of water organized in multilayers, while the elastic properties of HPC films seemed to be strongly dependent on the total concentration of sorbed water, regardless of its molecular organization on the surface of the biopolymer films.

To understand why the water effect on the elastic properties of the HPC films is considerably different from those of cassava and gelatin films, the polymer−water interaction should be discussed. The different nature of water−biopolymer interactions for studied biopolymer brought another element into this study. In the case of gelatin, the polypeptide chains are arranged in space in a way that the hydrophobic amino acids form a globule nucleus and the hydrophilic amino acids are located on the surface. During the first two stages of the hydration involving the strongly and weakly bound water, the polypeptide chains get stabilized from outside and inside, respectively. Our results showed that at these stages of hydration the bound water did not affect the elastic properties of the biopolymer structure and the α-peptide hydrogen bonds still control the rigidity of the gelatin network. However, the presence of the water in multilayers, which introduces water−water interactions, has a strong impact on the elasticity of the physical cross-linked network.

A similar mechanism occurred during the binding of the water molecules to the starch network. At low water content, the water molecules created hydrogen bonds with hydroxyl groups of starch; these first layers of water molecules already allowed the motion of the chains.[65] According to our results, these motions were not strong enough to be perceived on the global mechanical behavior of the starch films. This fact is probably due to the strength of the intermolecular interactions such as entanglements and physical cross-links, which restrict the chain motions introduced by bound water. The increase in humidity ratio brings more water molecules, which establish multilayers of water by bonding with the first water molecules already present in the media. It was suggested that these additional layers of water contribute to the increase in the mobility of the starch chains.[65] This disintegration of the starch chain interactions could explain the decrease in the global elasticity of the biopolymer network related to the appearance of the multilayer water.

In contrast to gelatin and cassava, HPC (type LF) with its high degree of hydroxypropyl substitution has only a few available hydrophilic sites to create a strong interaction with water molecules. According to the fit of the sorption isotherm with both models, the sorbed water molecules by the HPC network directly bind to the hydrophobic sites and form a monolayer of weakly bound water (low monolayer enthalpy of sorption, C, for the GAB model and low affinity for strong binding sites, K', for the D'Arcy and Watt model). It is interesting to underline that, in contrast to starch and gelatin, the presence of the weakly bound water and the formation of the biopolymer−hydrogen bonds contributed to the changes in the elasticity of the HPC network. This means that the hydrogen-bond interactions between two HPC highly substituted HPC molecules are also weak and could be easily affected by bound water. In addition, the measurements of the storage modulus by DMTA under dynamic hydration did not depict the appearance of multilayers of water molecules, which suggested that, contrary to the gelatin and cassava, the elasticity of the HPC network decreased with an increase in the total water content, independently of the water molecules' conformation on the surface of the HPC films.

Intrinsic Stiffness of Studied Biopolymers. To understand this difference in the elastic properties of the studied biopolymers, their stiffness at different structural levels should be discussed. First, the stress response of a single filament composed from the single macromolecule (intrinsic stiffness) and that of a network composed of individual molecules connected to each other in the absence of water molecules (dry state) should be compared. To evaluate the intrinsic stiffness of the single filament, the persistence length l_p (the persistence length is a characteristic of the stiffness of a chain in the limit of infinite chain length; for a filament, the length at which the molecule is able to bend significantly in two independent directions), determined from the intrinsic viscosity of the biopolymer solutions (in our case aqueous solution), could be used.[66] The persistence length of high-amylose starches molecules in DMSO/water solution (the values for persistence length for starches in aqueous solution were poorly reported in the literature) was reported to be from 6.6 to 8.1 nm (in this case, the persistence length of the starches reflected the stiffness of amylose chains in DMSO),[67] for HPC from 13 to 21 nm in aqueous solution[68] and finally for the gelatin from 2 to 2.5 nm in aqueous solution,[69] which makes the gelatin molecules more flexible in the dilute solution as compared to starch and HPC molecules. It was also reported that the conformation of starches in dilute solutions could be described as a random coil and the starches possess a high degree of intrinsic rigidity.[67] The behavior of the HPC molecules was reported to be intermediate between rod-like molecules and random coil, which makes it positioned between the flexible and rigid-chain polymers.[68]

On the other hand, the network formed by HPC macromolecules known as mesophasic conformation is considered as semiflexible.[70,71] Furthermore, the studied gelatin films, which present the network of physically cross-linked macromolecules, are believed to behave as a typical rigid chain polymer, which leads to its brittle behavior regardless of molecular conformations in the absence of a solvent such as water.[24]

This discrepancy between the stiffness of the single molecule and the physically cross-linked biopolymer network indicated a predominant role of the intermolecular interactions contributing to the global rigidity of the studied biopolymers. It is a well-known fact that when the biopolymer network is subjected to the stress, the elasticity response is strongly dependent on the cross-link density.[72] Qualitative consideration of the theory of rubber elasticity, which states that the elasticity modulus is inversely related to the average molar mass of elastically effective chains between the cross-links,[73] suggested that the gelatin and cassava starch networks are more densely cross-linked than the HPC. The B-type gelatin used in this study has a high molecular weight of 142 000 g/mol and a high bloom index (225 units), which corresponds to the high strength of its gel. We demonstrated previously that gelatin films cast from an aqueous solution did not display a significant plastic deformation and broke when the stress reached the yielding point.[22] This probably occurred because of the high density of physical cross-links, which are very strong and remained largely intact even when a high stress was applied.

In contrast, in the present study, the HPC films exhibited the lowest elasticity modulus and the largest plastic deformation as compared to gelatin and cassava starch films (as we demonstrated previously[23]), due to the weak intermolecular interactions, which are mobile enough to rearrange or disintegrate under sufficient high stress to allow permanent deformation or plasticity. On the other hand, the structural mobility of the HPC network could be explained by the flexibility of the HPC skeleton chain related to the secondary cyclization of the macromolecular structure by intramolecular hydrogen bonds rather than by steric hindrance due to the size of the substituting group.[4]

In the case of cassava starch, the low content of amylose, which has a high ability to interact by hydrogen bounds, resulted in relatively fewer intermolecular interactions as compared to high amylose content starches. The absence of crystalline structures meant that there were no permanent cross-links, but only a multitude of rather random intermolecular hydrogen bonds.

Finally, the global structural organization resulted in the liquid crystalline order found in the HPC films, and the ghost structure in the starch films and the crystalline order in the gelatin films also contributed to the global elastic properties of the chosen biopolymer films.

Prediction of Water Effect on Elastic Properties of Biopolymer Films. The analysis of the effect of water content on the elastic properties of the biopolymers films led us to suggest that this effect could be described by two simple models.

For HPC films, the results obtained for the elasticity modulus, with both techniques (DMTA and TA) as a function of the water content, showed that the softening of the films due to the water sorption is strongly dependent on the total water content of the films. The analysis of the experimental data suggested that the

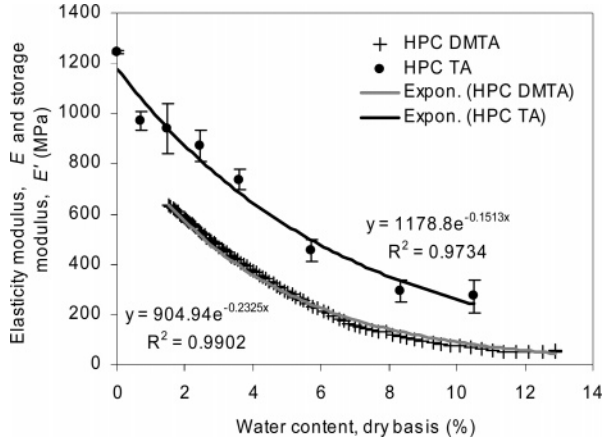

Figure 9. Storage modulus obtained by DMTA at 1 Hz and elastic modulus from TA tests for HPC films correlated to the water content (approximated exponential fit in solid lines).

elasticity modulus could be approximated mathematically as an exponential decay function of the total water content. Hence, the water content dependence of the elastic properties for HPC films could be expressed as:

$$E = E_0 e^{-aw} \tag{6}$$

where E is the elasticity modulus, E_0 is the elasticity modulus of the dry film, a is the decay constant, and w is the water content of the films. To validate the proposed model, the experimental DMTA and TA data for HPC films (Figure 8) was fitted with the exponential decay function (Figure 9).

It can be observed that the exponential decay function fitted the experimental data well, which is illustrated by the high correlation coefficient ($R^2 > 0.99$). The fit of the experimental data obtained by DMTA gave $E_0 = 905$ and the decay constant $a = 0.23$. On the other hand, the fit of the TA data produced different values of $E_0 = 1179$ and $a = 0.15$ due to the difference in the hydration of the films in the two procedures. According to the fitted data, the decay rate ($1/a$), which corresponds to a water content value, was equal to 4.3 and 6.7 for the DMTA and TA fit, respectively. The HPC isotherm sorption fit by the Darcy and Watt model (although the GAB model gave a better fitting of the HPC sorption isotherm, the prediction of the water content in the monolayer and multilayer by the D'Arcy and Watt model offered a better physical meaning for all studied biopolymer films (including HPC), and thus it was used for the analysis of the multilayer water content) (Figure 7c) showed that $1/a$ values could be interpreted as a critical water content at which the water molecules arranged in multilayers begin to affect the elastic properties of the biopolymer films.

In contrast to HPC films, the water dependence of the storage modulus (DMTA results) obtained during the dynamic hydration of cassava and gelatin films suggested that their elastic properties seemed to be correlated with the appearance of water in multilayers (Figure 8). Therefore, to demonstrate that the elasticity modulus could be related to the multilayer water content, the storage modulus measured by DMTA and elastic modulus calculated using the $\sigma = f(\epsilon)$ curves from TA tests were plotted as a function of the multilayer water content obtained from the fit of the sorption isotherm with the D'Arcy and Watt model (Figure 10). These plots suggested that the elastic modulus of gelatin and cassava films could be described in the first approximation as linearly dependent on the multilayer water content:

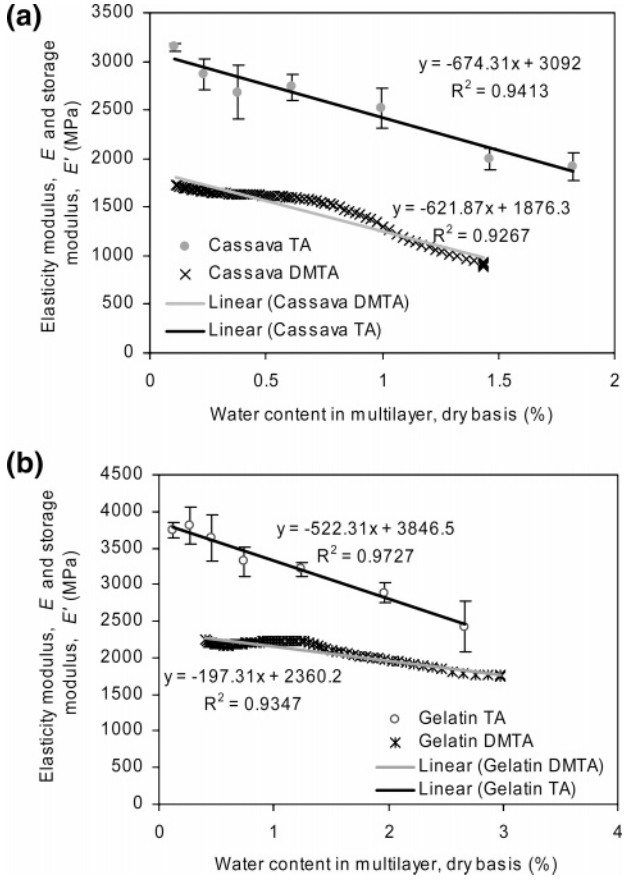

Figure 10. Storage modulus obtained by DMTA at 1 Hz and elastic modulus from TA tests for cassava starch (a) and gelatin (b) films correlated to the water content in multilayer (approximated linear fit in solid lines).

$$E = E_0 - bw_m \tag{7}$$

where E is the elasticity modulus, E_0 is the elasticity modulus of the dry films, b is a constant, and w_m is the multilayer water content calculated from the sorption isotherm fitting with respect to the water activity, a_w (RH/100). The constant b could be related to the rate of elasticity loss with respect to multilayer water content.

The nominal values of b obtained through the fit of the experiment values for cassava films were similar for dynamic and static hydration (Figure 10a). However, the slope of the linear fit for gelatin films was much lower in dynamic hydration ($b = 197$) than in static hydration ($b = 522$) (Figure 10b). This effect could be related to the difficulty to equilibrate the gelatin films at a given relative humidity during a short period of time, which was observed during the DVS experiments. On the other hand, the cassava films demonstrated a faster equilibration of the water content at a given relative humidity than gelatin films, which was expressed in the similar b values for both types of hydration.

Conclusions

The present study showed that the biopolymer films prepared from aqueous solutions of HPC, gelatin, and cassava starch formed complex networks at different structural levels. The peculiarities of these networks, their different molecular structures and interactions, had a strong influence their elastic properties. The three systems differ widely in the way adsorbed water interacts with these structures, and these different effects

of water on the biopolymer films could be used to explain the change of their elastic properties.

It was demonstrated that the elastic properties of the starch and gelatin films were strongly dependent on the appearance of water molecules arranged in multilayers and the elastic properties of the HPC films were affected by whole water content due to their different water binding ability. Two models were suggested to describe the influence of the water content on the elasticity modulus of the biopolymers. For HPC films, an exponential decay fit of the modulus as a function of the whole water content was accurate to describe their behavior because of the insignificant amount of strong bound water in this biopolymer. For cassava and gelatin films, a linear fit of the modulus as a function of the water arranged in multilayers showed a strong correlation.

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References and Notes

(1) Bigi, A.; Panzavolta, S.; Rubini, K. Relationship between triple-helix content and mechanical properties of gelatin films. *Biomaterials* **2004**, *25*, 5675−5680.
(2) Bigi, A.; Borghi, M.; Cojazzi, G.; Fichera, A. M.; Panzavolta, S.; Roveri, N. Structural and mechanical properties of crosslinked drawn gelatin films. *J. Therm. Anal. Calorim.* **2000**, *61*, 451−459.
(3) Dai, C.; Liu, M. The effect of crystallinity and aging enthalpy on the mechanical properties of gelatin films. *Mater. Sci. Eng., A* **2006**, *423*, 121−127.
(4) Evmenenko, G.; Yu, C. J.; Kewalramani, S.; Dutta, P. Structural reorganization in films of cellulose derivatives in the presence of colloidal particles. *Polymer* **2004**, *45*, 6269−6273.
(5) Mali, S.; Grossmann, M. V. E.; Garcia, M. A.; Martino, M. N.; Zaritzky, N. E. Effects of controlled storage on thermal, mechanical and barrier properties of plasticized films from different starch sources. *J. Food Eng.* **2006**, *75*, 453−460.
(6) Mali, S.; Sakanaka, L. S.; Yamashita, F.; Grossmann, M. V. E. Water sorption and mechanical properties of cassava starch films and their relation to plasticizing effect. *Carbohydr. Polym.* **2005**, *60*, 283−289.
(7) Rindlav-Westling, A.; Stading, M.; Hermansson, A. M.; Gatenholm, P. Structure, mechanical and barrier properties of amylose and amylopectin films. *Carbohydr. Polym.* **1998**, *36*, 217−224.
(8) Seidel, A.; Liivak, O.; Calve, S.; Adaska, J.; Ji, G.; Yang, Z.; Grubb, D.; Zax, D. B.; Jelinski, L. W. Regenerated spider silk: Processing, properties, and structure. *Macromolecules* **2000**, *33*, 775−780.
(9) Shingel, K. I.; Faure, M. P. Structure-property relationships in poly-(ethylene glycol)-protein hydrogel systems made from various proteins. *Biomacromolecules* **2005**, *6*, 1635−1641.
(10) Sorlier, P.; Denuziere, A.; Viton, C.; Domard, A. Relation between the degree of acetylation and the electrostatic properties of chitin and chitosan. *Biomacromolecules* **2001**, *2*, 765−772.
(11) Dobrzynski, P.; Li, S. M.; Kasperczyk, J.; Bero, M.; Gasc, F.; Vert, M. Structure-property relationships of copolymers obtained by ring-opening polymerization of glycolide and ϵ-caprolactone. Part 1. Synthesis and characterization. *Biomacromolecules* **2005**, *6*, 483−488.
(12) Angellier, H.; Molina-Boisseau, S.; Lebrun, L.; Dufresne, A. Processing and structural properties of waxy maize starch nanocrystals reinforced natural rubber. *Macromolecules* **2005**, *38*, 3783−3792.
(13) Larotonda, F. D. S.; Matsui, K. N.; Soldi, V.; Laurindo, J. B. Biodegradable films made from raw and acetylated cassava starch. *Braz. Arch. Biol. Technol.* **2004**, *47*, 477−484.
(14) Phan, T. D.; Debeaufort, F.; Luu, D.; Voilley, A. Functional properties of edible agar-based and starch-based films for food quality preservation. *J. Agric. Food Chem.* **2005**, *53*, 973−981.
(15) Tonukari, N. J. Cassava and the future of starch. *Biotechnol. Issues Dev. Countries* **2004**, *7*, 5−8.
(16) Alvarez-Lorenzo, C.; Gomez-Amoza, J. L.; Martinez-Pacheco, R.; Souto, C.; Concheiro, A. Interactions between hydroxypropylcelluloses and vapour/liquid water. *Eur. J. Pharm. Biopharm.* **2000**, *50*, 307−318.
(17) Chang, Y. P.; Abd Karim, A.; Seow, C. C. Interactive plasticizing-antiplasticizing effects of water and glycerol on the tensile properties of tapioca starch films. *Food Hydrocolloids* **2006**, *20*, 1−8.
(18) Kent, M.; Meyer, W. Complex permittivity spectra of protein powders as a function of temperature and hydration *J. Phys. D: Appl. Phys.* **1984**, *17*, 1687−1698.
(19) Kristo, E.; Biliaderis, C. G.; Zampraka, A. Water vapour barrier and tensile properties of composite caseinate-pullulan films: Biopolymer composition effects and impact of beeswax lamination. *Food Chem.* **2007**, *101*, 753−764.
(20) Stubberud, L.; Arwidsson, H. G.; Larsson, A.; Graffner, C. Water solid interactions II. Effect of moisture sorption and glass transition temperature on compactibility of microcrystalline cellulose alone or in binary mixtures with polyvinyl pyrrolidone. *Int. J. Pharm.* **1996**, *134*, 79−88.
(21) Van den Berg, C.; Bruin, S. *Water activity: influence on food quality*; Academic Press: New York, 1981; pp 1−62.
(22) Yakimets, I.; Wellner, N.; Smith, A. C.; Wilson, R. H.; Farhat, I.; Mitchell, J. Mechanical properties with respect to water content of gelatin films in glassy state. *Polymer* **2005**, *46*, 12577−12585.
(23) Yakimets, I.; Wellner, N.; Smith, A. C.; Wilson, R. H.; Farhat, I.; Mitchell, J. Effect of water content on the fracture behaviour of hydroxypropyl cellulose films studied by the essential work of fracture method. *Mech. Mater.* **2007**, *39*, 500−512.
(24) Kozlov, P. V.; Burdygina, G. I. The structure and properties of solid gelatin and the principles of their modification. *Polymer* **1983**, *24*, 651−666.
(25) Pal, K.; Banthia, A. K.; Majumdar, D. K. Polyvinyl alcohol-gelatin patches of salicylic acid: Preparation, characterization and drug release studies. *J. Biomater. Appl.* **2006**, *21*, 75−91.
(26) Giraudier, S.; Hellio, D.; Djabourov, M.; Larreta-Garde, V. Influence of weak and covalent bonds on formation and hydrolysis of gelatin networks. *Biomacromolecules* **2004**, *5*, 1662−1666.
(27) Crescenzi, V.; Francescangeli, A.; Taglienti, A. New gelatin-based hydrogels via enzymatic networking. *Biomacromolecules* **2002**, *3*, 1384−1391.
(28) Bigi, A.; Bracci, B.; Cojazzi, G.; Panzavolta, S.; Roveri, N. Drawn gelatin films with improved mechanical properties. *Biomaterials* **1998**, *19*, 2335−2340.
(29) Hizukuri, S.; Takeda, Y.; Yasuda, M.; Suzuki, A. Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* **1981**, *94*, 205−213.
(30) Lourdin, D.; Valle, G. D.; Colonna, P. Influence of amylose content on starch films and foams. *Carbohydr. Polym.* **1995**, *27*, 261−270.
(31) Derek, R.; Prentice, M.; Stark, J. R. Granule residues and "ghosts" remaining after heating A-type barley-starch granules in water. *Carbohydr. Res.* **1992**, *227*, 121−130.
(32) Li, J. H.; Vasanthan, T.; Hoover, R.; Rossnagel, B. G. Starch from hull-less barley: IV. Morphological and structural changes in waxy, normal and high-amylose starch granules during heating. *Food Res. Int.* **2004**, *37*, 417−428.
(33) Smits, A. L. M.; Kruiskamp, P. H.; van Soest, J. J. G.; Vliegenthart, J. F. G. The influence of various small plasticisers and malto-oligosaccharides on the retrogradation of (partly) gelatinised starch. *Carbohydr. Polym.* **2003**, *51*, 417−424.
(34) Petersson, M.; Loren, N.; Stading, M. Characterization of phase separation in film forming biopolymer mixtures. *Biomacromolecules* **2005**, *6*, 932−941.
(35) Rindlav-Westling, A.; Gatenholm, P. Surface composition and morphology of starch, amylose, and amylopectin films. *Biomacromolecules* **2003**, *4*, 166−172.
(36) Rindlav-Westling, A.; Stading, M.; Gatenholm, P. Crystallinity and morphology in films of starch, amylose and amylopectin blends. *Biomacromolecules* **2002**, *3*, 84−91.
(37) Werbowyj, R. S.; Gray, D. G. Liquid crystalline structure in aqueous hydroxypropylcellulose solution. *Mol. Cryst. Liq. Cryst.* **1976**, *34*, 97−103.
(38) Wirick, M. G.; Waldman, M. H. Some solution properties of fractionated water soluble hydroxypropylcellulose. *J. Appl. Polym. Sci.* **1970**, *14*, 579−597.
(39) Yamagishi, T.-A.; Sixou, P. Preparation and characteristics of cholesteric gel from pentyl ether of hydroxypropyl cellulose. *Polymer* **1995**, *36*, 2315−2317.
(40) Rials, T. G.; Glasser, W. G. Thermal and dynamic mechanical properties of hydroxylpropyl cellulose film. *J. Appl. Polym. Sci.* **1988**, *36*, 749−758.
(41) Wang, J.; Labes, M. M. Control of the anisotropic mechanical properties of liquid crystal polymer films by variations in their bended texture. *Macromolecules* **1992**, *25*, 5790−5793.

(42) Bizot, H. Using the 'GAB' model to construct sorption isotherms. In *Physical properties of foods*; Al, J. e., Ed.; Applied Science: New York, 1983; pp 43−54.
(43) Henderson, S. M. A basic concept of equilibrium moisture. *Agric. Eng.* **1952**, *33*, 29−32.
(44) Oswin, C. R. The kinetics of package life III. The isotherms. *J. Chem. Ind.* **1946**, *65*, 419−421.
(45) Biliaderis, C. G.; Lazaridou, A.; Arvanitoyannis, I. Glass transition and physical properties of polyol-plasticised pullulan-starch blends at low moisture. *Carbohydr. Polym.* **1999**, *40*, 29−47.
(46) Zhou, S.; Tashiro, K.; Hongo, T.; Shirataki, H.; Yamane, C.; Ii, T. Influence of water on structure and mechanical properties of regenerated cellulose studied by an organized combination of infrared spectra, X-ray diffraction and dynamic viscoelastic data measured as functions of temperature and humidity. *Macromolecules* **2001**, *34*, 1274−1280.
(47) Wulansari, R.; Mitchell, J. R.; Blanshard, J. M. V.; Paterson, J. L. Why are gelatin solutions Newtonian? *Food Hydrocolloids* **1998**, *12*, 245−249.
(48) Mestres, C.; Matencio, F.; Pons, B.; Yajid, M.; Fliedel, G. A rapid method for the determination of amylose content by using Differential Scanning Calorimetry. *Starch/Staerke* **1996**, *48*, 2−6.
(49) Šašić, S.; Ozaki, Y. Statistical two-dimensional correlation spectroscopy: Its theory and applications to sets of vibration spectra. *Anal. Chem.* **2001**, *73*, 2294−2301.
(50) Rizvi, S. S. H. Thermodynamic properties of foods in dehydration. In *Engineering properties of foods*, 2nd ed.; Rao, M. A., Rizvi, S. S. H., Eds.; Marcel Dekker, Inc.: New York, 1994; pp 223−310.
(51) D'Arcy, R. L.; Watt, I. C. Analysis of sorption isotherms of non-homogeneous sorbents. *Trans. Faraday Soc.* **1970**, *66*, 1236−1245.
(52) Maleev, V.; Semenov, M.; Kruglova, E.; Bolbukh, T.; Gasan, A.; Bereznyak, E.; Shestopalova, A. Spectroscopic and calorimetric study of DNA interaction with a new series of actinocin derivatives. *J. Mol. Struct.* **2003**, *645*, 145−158.
(53) Lomauro, G. J.; Bakshi, A. S.; Labuza, T. P. Evaluation of food moisture sorption isotherms equations. Part I: Fruit, vegetables and meat products. *Lebensm.-Wiss. Technol.* **1985**, *18*, 111−117.
(54) Perez-Alonso, C.; Beristain, C. I.; Lobato-Calleros, C.; Rodriguez-Huezo, M. E.; Vernon-Carter, E. J. Thermodynamic analysis of the sorption isotherms of pure and blended carbohydrate polymers. *J. Food Eng.* **2006**, *77*, 753−760.
(55) Noda, I. Advances in two-dimensional correlation spectroscopy. *Vib. Spectrosc.* **2004**, *36*, 143−165.
(56) Noda, I.; Dowrey, A. E.; Marcott, C.; Story, G. M.; Ozaki, Y. Generalized two-dimensional correlation spectroscopy. *Appl. Spectrosc.* **2000**, *54*, 236A−248A.
(57) Samuels, R. J. Solid-state characterization of structure and deformation behavior of water-soluble hydroxypropylcellulose. *J. Polym. Sci., Part A: Polym. Chem.* **1969**, *7*, 1197, 1969.
(58) Wilkers, G. L.; The Vu, B. *Superstructure in films of bio and biorelated polymers as noted by small angle light scattering. Structure and properties of polymer films*; Plenum Press: New York−London, 1973.
(59) Werbowyj, R. S.; Gray, D. G. Cholesteric structure in concentrated hydroxypropyl cellulose solutions. *Abstr. Pap. Am. Chem. Soc.* **1979**, 101−101.
(60) Rials, T. G.; Glasser, W. G. Multiphase materials with lignin. IV Blends of hydroxypropyl cellulose with Lignin. *J. Appl. Polym. Sci.* **1989**, *37*, 2399−2415.
(61) Suto, S.; Suzuki, K. Crosslinked hydroxypropyl cellulose films retaining cholesteric liquid crystalline order: 2. Anisotropic swelling behaviour in water. *Polymer* **1997**, *38*, 391−396.
(62) Atkin, N. J.; Abeysekera, R. M.; Robards, A. W. The events leading to the formation of ghost remnants from the starch granule surface and the contribution of the granule surface to the gelatinization endotherm. *Carbohydr. Polym.* **1998**, *36*, 193−204.
(63) Gomez-Carracedo, A.; Alvarez-Lorenzo, C.; Gomez-Amoza, J. L.; Concheiro, A. Chemical structure and glass transition temperature of non-ionic cellulose ethers DSC, TMDSC (R) - Oscillatory rheometry study. *J. Therm. Anal. Calorim.* **2003**, *73*, 587−596.
(64) Lee, J. L.; Pearce, E. M.; Kwei, T. K. Side-Chain crystallization in alkyl-substituted semiflexible polymers. *Macromolecules* **1997**, *30*, 6877−6883.
(65) Brouillet-Fourmann, S.; Carrot, C.; Lacabanne, C.; Mignard, N.; Samouillan, V. Evolution of interactions between water and native corn starch as a function of moisture content. *J. Appl. Polym. Sci.* **2002**, *86*, 2860−2865.
(66) Yamakawa, H.; Yoshizaki, T. Transport coefficients of helical wormlike chains 3. Intrinsic viscosity. *Macromolecules* **1980**, *13*, 633−643.
(67) Carriere, C. J.; Bagley, E. B. Calculation of the persistence length of solubilized starches from intrinsic viscosity measurements. *J. Rheol.* **1999**, *43*, 753−763.
(68) Werbowyj, R. S.; Gray, D. G. Ordered phase formation in concentrated hydroxypropylcellulose solutions. *Macromolecules* **1980**, *13*, 69−73.
(69) Mohanty, B.; Aswal, V. K.; Kohlbrecher, J.; Bohidar, H. B. Length scale hierarchy in sol, gel, and coacervate phases of gelatin. *J. Polym. Sci., Part B: Polym. Phys.* **2006**, *44*, 1653−1665.
(70) Viney, C.; Putnam, W. S. The banded microstructure of sheared liquid-crystalline polymers. *Polymer* **1995**, *36*, 1731−1741.
(71) Shinichi, S.; Masataka, K.; Mikio, K. Static tensile and dynamic mechanical properties of hydroxypropylcellulose films prepared under various conditions. *J. Appl. Polym. Sci.* **1986**, *31*, 1327−1341.
(72) Gluck-Hirsch, J. B.; Kokini, J. L. Determination of the molecular weight between crosslinks of waxy maize starches using the theory of rubber elasticity. *J. Rheol.* **1996**, *41*, 129−140.
(73) Flory, P. J. Molecular theory of rubber elasticity. *Polymer* **1979**, *20*, 1317−1320.

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