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Effects of Different Moisture Contents on Physical Properties of PVA-Gelatin Films

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Abstract In this work, it was investigated the effect of different moisture contents on PVA-gelatin films by means of dielectric properties, infrared spectroscopy, microwave response and gravimetric method. The films were elaborated from a blend of gelatin and PVA, with 0 and 25 % glycerol. The sorption isotherms were determined by gravimetric methods, at 25 °C. A capacitor was used for dielectric measurements, and a device called SOLFAN setup was used for microwave measurements. The sorption isotherms were markedly affected by the glycerol content and relative humidity, due to the hygroscopic nature of the films. The dielectric constant and the microwave response signal were also strongly affected by the moisture and glycerol content in the films. Finally, Infrared spectra showed some changes in the amide peak positions, attributed to the modifications in the interactions between the macromolecules. The behaviors obtained in this work were explained on the basis the way the water enters in the film matrix.

Keywords Biodegradable film · Sorption isotherm · Dielectric · Infrared · Moisture content

Introduction

Biodegradable films are materials elaborated from natural biopolymers, such as polysaccharides and proteins,¹ and thus, can be used as alternative materials for food packaging, food recovering, among other applications. Due to the hygroscopic nature of these films, they possess high sensibility to environmental conditions, including the relative

humidity² and origin of the raw material.³ To improve some relevant properties of these films (flexibility, gas and vapor barrier, etc.), some additives including oils extracted from cloves⁴ and plasticizers^{5,6} are added to the film formulation. However, some plasticizers such as glycerol contain hydrophilic groups, in which the water molecules interact with the macromolecules by means of hydrogen bonds, resulting in moisture absorption. Water molecules can constitute a problem due to their strong plasticizer effects in films, modifying the physical properties of the material.² Thus, the qualitative or quantitative determination of moisture content in films is always necessary and valuable. A possible means to minimize the problem of the moisture content in these films is the association of them with some synthetic polymers through blending, such as poly(vinyl alcohol) (PVA). Polymer blending is a technique widely applied in polymer science to obtain materials with improving properties. Several studies have been conducted using blends of poly(vinyl alcohol) (PVA) and gelatin in order to improve the physical and functional properties of edible films.⁷

Poly (vinyl alcohol) is a synthetic polymer, water soluble, and highly hydrophilic, and thus, biodegradable and biocompatible material. Due to its characteristics, PVA has been used in cosmetic, food packaging materials, pharmaceutical and medical applications, and more important, possesses high ability of film formation. Poly (vinyl alcohol) is obtained by polymerization of vinyl acetate, followed by partial hydrolysis of the resulting ester in the presence of an alkaline catalyst. The number of acetate groups in polyvinyl alcohol is determined by the degree of hydrolysis.

Some alternative methods can be used to characterize gelatin films.^{8,9} Dielectric properties⁵ and microwave methods provide valuable information on the structural properties of materials, together to that obtained from infrared spectroscopy and gravimetric methods. These methods are useful to study the interactions that occur in the film structure,

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based on their composition and temperature. In the case of natural polymers, the dielectric properties can arise mainly from hydrogen bonds and polar structures present in the macromolecule.^{10,11} Free water also contributes strongly to the dielectric response. The large size of the macromolecular chains affects markedly the relaxation time, decreasing the dielectric response at higher frequencies.¹⁰ Some works have been reported in literature, describing water activity around biopolymers, by means of microwave methods that use the time-domain reflectometry method.^{11–13}

The dielectric properties of the materials depend on different factors. In hygroscopic materials such as foods and edible films, the amount of water in the material is generally a dominant factor. The dielectric properties depend on the frequency of the applied alternating electric field, the temperature of the material, and on the density, composition, and structure of the material. In granular or particulate materials, the bulk density of the air–particle mixture is another factor that influences the dielectric properties. In addition, the dielectric properties of materials are dependent on their chemical composition and especially on the permanent dipole moments associated with water and any other molecules making up the material of interest.¹⁴

When stored at different relative humidity conditions, the water transport phenomenon occurs in the films, and as a consequence, they gain or loss moisture as a function of relative humidity present in the environment, and therefore, the physical properties of the films become strongly affected by the water content. The way the water molecules enter in the film matrix plays important role on structural properties of gelatin films, including changes in the polarizability mechanisms (Functional polar groups, water molecules, chemical bounds, etc.) and modifications in the interactions between macromolecules and water, depending on the moisture content, and therefore, can be evaluated by different methods, including infrared spectroscopy, dielectric measurements, sorption isotherms and microwave methods. For this study, it was chosen gelatin-PVA blended film samples with and without glycerol. Measurements were performed at 25 °C.

Materials and Methods

Materials

The films studied in this work were prepared using a commercial pigskin gelatin (type A, bloom=242–248; molecular weight $\cong 5.2 \cdot 10^4$ Da; moisture content=9.3 %) provided by Gelita do Brasil Ltda (São Paulo, Brazil), and poly(vinyl alcohol) with a degree of hydrolysis (DH) of 88 %,

molecular weight of 31–50 kDa (Celvol® 504) donated by Celanese Ltd (Dallas, USA). Glycerol (Synth, Brazil) was used as plasticizer.

Film Production

The films were produced by casting a solution containing a mixture of 2 g macromolecules (1 g gelatin, 1 g PVA) and 100 g distilled water, plus 0 or 25 g glycerol/100 g macromolecules. The gelatin was first hydrated by a period of 30 min., and then, dissolved at 55 °C in 50 g water, using a thermostatic water-bath (Marconi, Model TE 184). Then, glycerol was added and the solution was held at 55 °C for further 15 min under constant stirring. At the same time, PVA was first homogenized in 50 g distilled water and then dissolved at 90 °C over magnetic stirring (Tecnal-TE085) by a period of 30 min. Then, the solutions (PVA and gelatin) were mixed and stirred by other 30 min. at room temperature in order to obtain the filmogenic solution. Finally, the filmogenic solution was poured in an acrylic plate and dehydrated in an oven with air circulation and renewal (Marconi, MA037), at 30 °C by 18–24 h, in order to obtain transparent and flexible films.

Pre-Conditioning of Samples

Films were preconditioned at 25 °C on desiccators containing different saturated salt solutions, in order to control the moisture content in the films. The following salts (Synth, São Paulo, Brazil) were chosen, in order to provide a water activity ranging from 0.11 to 0.85 [**1**-LiCl, **2**-MgCl₂, **3**-K₂CO₃, **4**-Mg(NO₃)₂, **5**-NaBr, **6**-NaCl, **7**-KCl, **8**-silica gel]. The preconditioning time required for equilibrium was around 5 weeks, based on weight variations of the samples expressed in dry basis, not exceeding 0.1 %. The moisture content of samples (*X*), expressed as $(g \text{ water})/(100 g \text{ dry solids})$ was determined by further drying at 105 °C for 24 h, and the respective weight loss.

After preconditioning, samples were immediately analyzed according to the methodology described as follows.

Dielectric Measurements

For dielectric constant measurements, it was used an ordinary electric capacitometer (mod. instrutherm CP-400). In this measurement, it was evaluated the capacitance of a cell formed by two small parallel metallic plates separated by a given distance, first, with the empty cell and then, with the sample inside the cell, as a function of moisture content in the film. The film samples were cut in small rectangles with area equal to the area of the plates. The distance between the metallic plates was taken as the thickness of the films. The

dielectric constant was obtained from the ratio between the capacitance containing the film, and the empty cell (Eq. 1).

$$\varepsilon' = \frac{C}{C_0} \quad (1)$$

where C is the electric capacitance of the cell containing the film and C_0 , the empty cell (air capacitance), given by the Eq. 2:

$$C_0 = \varepsilon_0 A d^{-1} \quad (2)$$

where ε_0 is a constant (8.85×10^{-12} F), A is the area of the metallic plates (m^2) and d^{-1} is the reciprocal of the distance between the plates (m^{-1}). The capacitance was evaluated at fixed frequency of 800 Hz. These measurements were performed at room conditions (23–25 °C and 45–55 % of relative humidity), in triplicate.

In this work, it was evaluated only the real part of the dielectric constant. Some difficulties arose in the measurements of ε'' , which depends on the electric conductivity of the films.

In order to explain the effect of the moisture content on the dielectric constant, two other experiments were performed: microwave analysis and Fourier transformed infrared spectroscopy, using always the same samples.

Microwave Analysis

For the microwaves analysis, performed at 10 GHz, a special device called SOLFAN (USA) setup was used. This device consists in a microwave Gunn source and a diode detector mounted in the same rectangular waveguide, acting as a resonant cavity, operating in the TE₁₀ mode (Transverse Electric field propagation mode). This set was attached to a horn antenna, acting as sensor.

To carry out these analyses, the films were placed over the aperture of the antenna, in order to receive the microwave signal. The area of the film was larger than the area of the antenna aperture.

The reflected microwave signal response, evaluated as a function of moisture content (free water domain), was collected directly from the detector and measured by ordinary methods, using a digital voltmeter (mV scale). Microwave power level was about 15 mW, which was found to be insufficient to induce any perceptible heating of the film.

Fourier Transformed Infrared Spectroscopy

Infrared spectra (FTIR) of films as a function of moisture content were recorded between 4,000 and 600 cm^{-1} at 4 cm^{-1} of resolution, with a Spectrum One (Perkin Elmer) spectrometer, supplied with a universal attenuated total reflectance (UATR) accessory.⁸ Measurements were performed at room temperature.

Results and Discussions

Dielectric Behaviors

The dielectric constant ε' of gelatin-PVA based films evaluated after different salt conditioning are plotted in Figure 1. For both films (with and without glycerol), a similar behavior was observed, despite the marked differences in dielectric strength. For lower moisture content, the dielectric constant presented a non-linear increasing. The lower strength of the first dielectric constant values is due to the lower polarizability (high relaxation time) of the bounded water–macromolecule structures. Beyond a given moisture content (threshold), the dielectric constant was found to increase linearly. This behavior can be probably attributed to the gradual conversion from bound water to free water, and the linear region, to the gradual increasing of free water in the monolayer and multilayer domain,¹⁵ and thanks to the high dipolar moment of free water molecule, it contributes markedly to the dielectric strength of the material.

The glycerol was found to affect strongly the strength of the dielectric properties, as well as, the value of the threshold of the moisture content (Xt). For films without glycerol, the Xt was close to 8.5 % (Figure 1a). And below this condition, the dielectric constant remained near 4. For X higher than Xt, the dielectric constant increased linearly

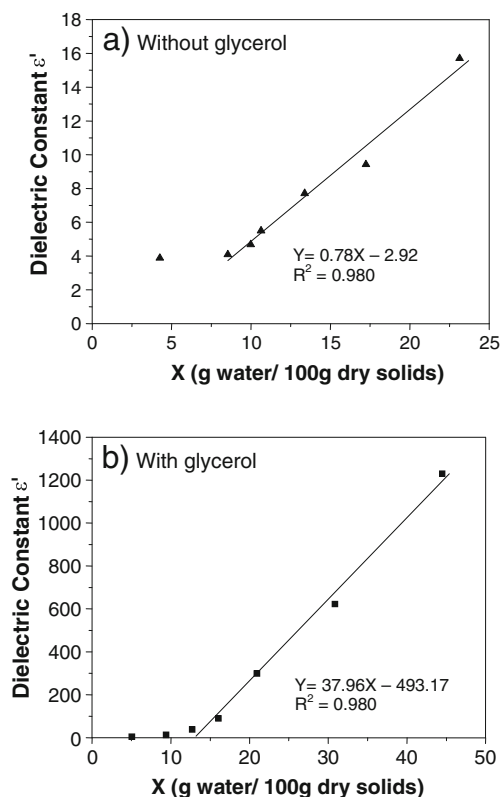


Fig. 1 Dielectric constant of gelatin-PVA based films **a** without glycerol and **b** with 25 % glycerol

from 4 to 16, as a function of the moisture content ranging between 8.5 and near 23 %. On the other hand, for films with glycerol (Figure 1b), X_t was evaluated as 12 %. Above this condition, the dielectric constant varied linearly from 50 to 1,250, for the moisture content ranging from 13 to 44 %. The domain of free water was, therefore, higher than that of films without glycerol, due to the intense hygroscopic character of this plasticizer. High dielectric constant values were also observed by Khutorsky and Lang,¹⁶ which obtained values of dielectric permittivity of the order of 10^4 at low frequencies, for triglycine sulfate (TGS) gelatin films conditioned at high relative humidity. According to these authors, this phenomenon could be caused possibly by the occurrence of an electric double layer on the TGS crystallite-gelatin phase interfaces. Thus, in the present study, it can be suggested that glycerol may promote high adsorption of water in the gelatin rich phase,² and thus, a double layer could be formed.

Sorption Isotherm

The samples were preconditioned in different salts until equilibrium conditions, and then, the sorption isotherms were determined; the results are plotted in the Figure 2. These sorption isotherms showed the typical sigmoid (Type II) shape, which is the result of water interaction with the film polymeric chains. Glycerol increased markedly the water vapor sorption due to its hydrophilic character, favoring the absorption of water molecules. Glycerol was added to the film forming solution to act as a plasticizer, increasing the flexibility of the material.

The GAB (Guggenheim-Anderson-De Boer) model (Eq. 3) was used to describe the sorption isotherms of films studied in this work ($R^2 \cong 0.99$).

$$X = \frac{X_m C_G K_G A_w}{(1 - K_G A_w)(1 - K_G A_w + C_G K_G A_w)} \quad (3)$$

Where: A_w = water activity; X_m = moisture content in monolayer; C_G, K_G = constants; and X = equilibrium moisture content.

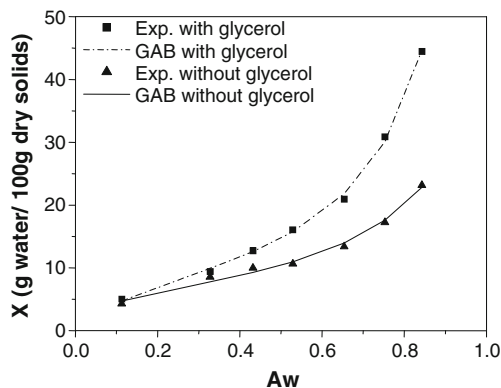


Fig. 2 Sorption isotherms of gelatin-PVA based films without and with glycerol

The GAB model was fitted to equilibrium data using the Statistica® software (Version 9.1; SAS Institute Inc., Cary, NC, USA) allowing the determination of the monolayer moisture content (X_m), which is of particular interest since it indicates the amount of water strongly absorbed in specific sites in the biopolymer. The values of monolayer moisture content obtained were 7.3 and 4.1 g water/100 g dry solids for PVA-gelatin based films with and without glycerol, respectively. These values were slightly lower than the X_m ^{17,18} for films based on gelatin with and without chemical treatment ($X_m \cong 12.5$ g/100 g dry solid).

Also, the value of K_G was calculated and found to be 0.86 for films without glycerol, and 0.96 for those with glycerol. The value of K_G provides a measure of the interactions between the molecules in multilayers with the absorbent, and tends to fall between the energy value of the molecules in the monolayer and that of liquid water.

Microwave Measurements

Contrarily to that observed from the dielectric measurements, a linear behavior was observed in the microwave signal response in the films, in the whole domain of moisture content studied (Figure 3). This behavior can be attributed to an increasing in the free water, i.e., in the monolayer and multilayer domain. This effect was more pronounced in films with glycerol, probably due to the dominance of water in the multilayer domain, in relation to that observed in the films without glycerol.

Microwave energy is strongly absorbed mainly by free water. The time elapsed of water bound to the macromolecule structure to achieve the equilibrium becomes lower than of free water¹³ and thus, the microwave energy becomes less absorbed by bound water than by free water.

Infrared Spectroscopy

The FTIR spectra of films made of PVA-gelatin with and without glycerol are shown in Figures 4 and 5, respectively.

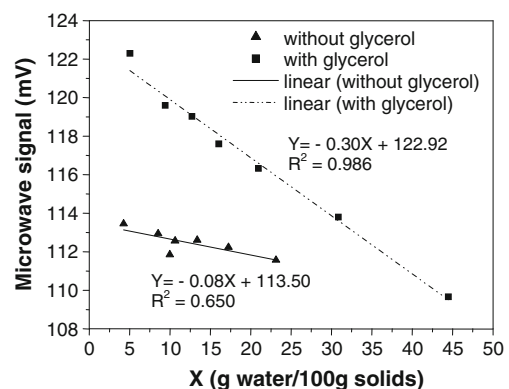
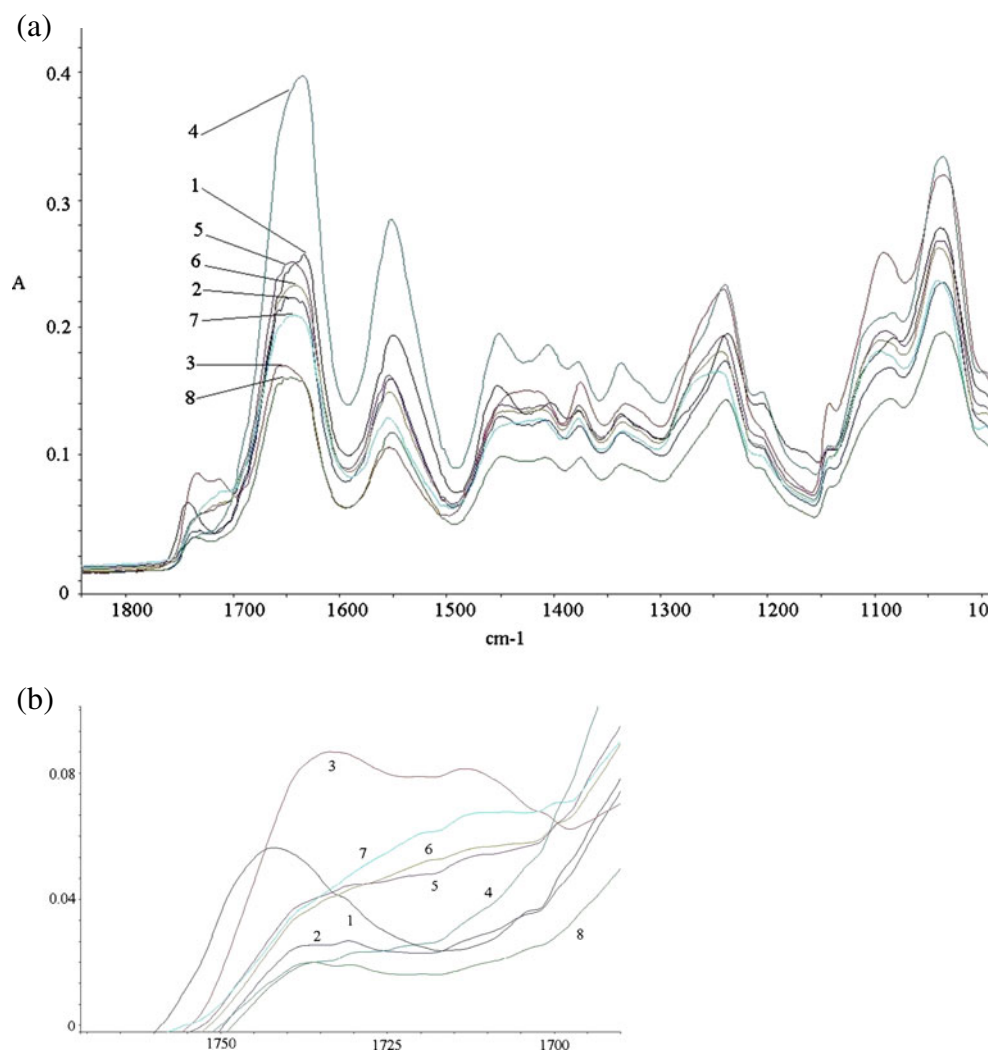


Fig. 3 Microwave signal response for gelatin-PVA based films

Fig. 4 **a** Examples of FTIR spectra of gelatin films containing PVA and glycerol, conditioned in different salts (represented by the numbers in each spectra); **b** Main changes near 1,730 cm⁻¹ due to moisture content

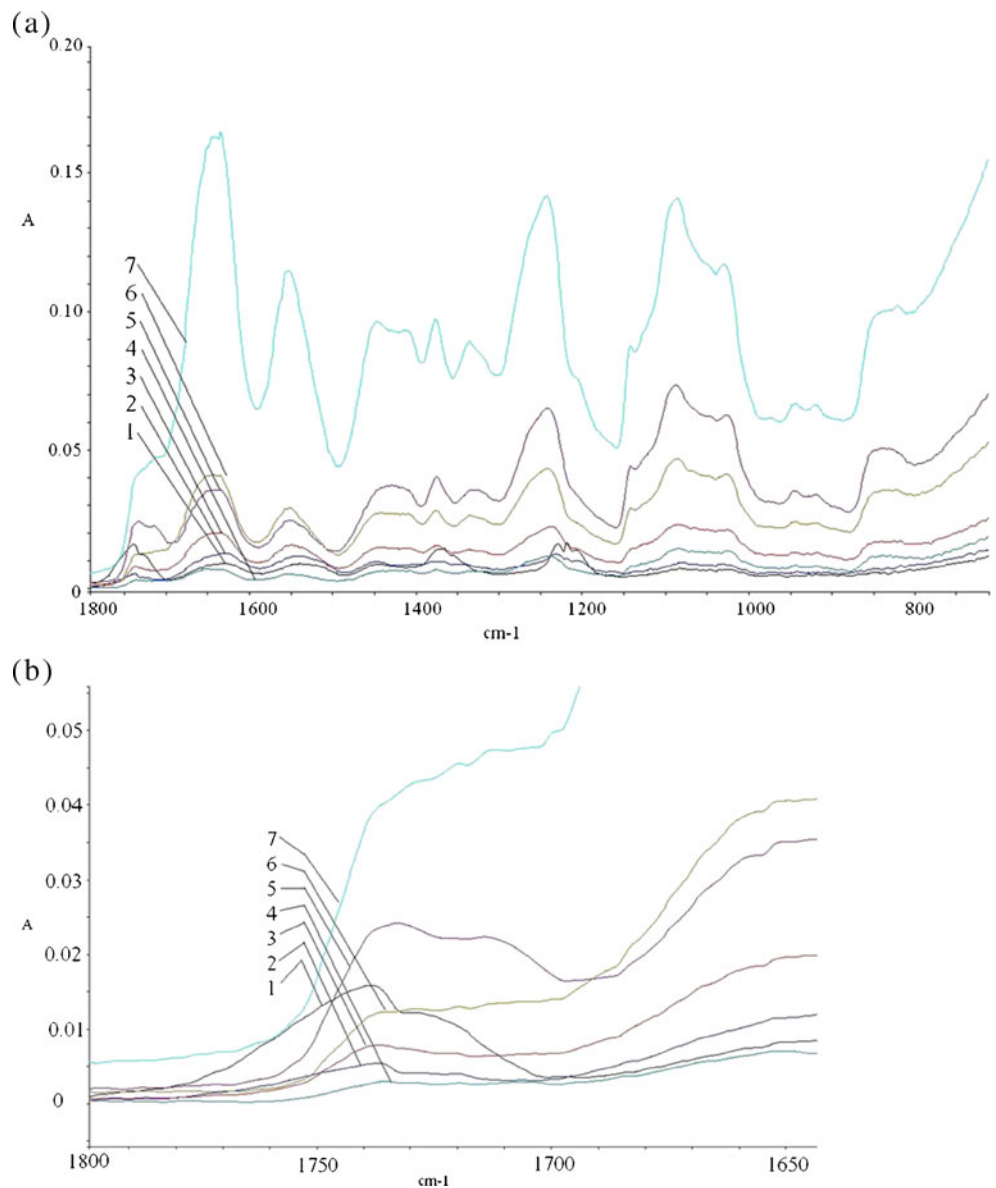


The main changes occur in the fingerprint region. FTIR spectra of samples display the absorption regions of the chemical groups associated with poly(vinyl alcohol) (–OH, –CO, –CH₂) and important functional groups of the gelatin (amides I, II and III). It is observed a C–H broad alkyl stretching band (2,850–3,000 cm⁻¹) and typical strong hydroxyl bands for free alcohol (non-bonded –OH stretching band at 3,600–3,650 cm⁻¹), and bonded hydrogen (3,200–3,570 cm⁻¹). Some slight changes between 1,700 and 1,750 cm⁻¹ can be observed in the spectra of the films as a function of moisture content, which can be due to the C=O and C–O stretching from acetate group remaining of PVA.¹⁹ In Figures 4b and 5b, the behavior in peak shape as a function of moisture content can be well visible, occurring a gradual decreasing in the peak near 1,740 cm⁻¹, in contrast to an increasing of a shoulder near 1,710 cm⁻¹. It can be seen a shoulder in the region near 1,720 cm⁻¹ for films without glycerol and low moisture content. This shoulder vanishes with moisture content increasing, in such a way that the peak shape becomes closer in both films with high moisture content. The variations in the peak intensities were, therefore, found to be sensitive to water content,

which possible interacts with the acetate group content in the film via C–O and C=O stretching bound.¹⁹ The bands situated at 3,288, 1,632 and 1,548 cm⁻¹ correspond to free water (or amide III), amide I and amide II, respectively. The amide I arises from stretching of C=O of proteins of amide; the amide II arises from vibrations N–H and stretching vibrations of C–N. The amide III corresponds to vibrations in plane of C–N and N–H of bound amide, and vibrations of CH₂ groups of glycine.^{20–22}

The amide II peak presented visible changes in its position, as a function of water content, as depicted in Figure 6, which seems to follow a given trend. The changes were found to be more prominent in the films without glycerol, mainly in the region of low water content. This behavior can possible be explained by the modifications in the interactions between the polymeric matrix, caused by the bound water, which can be accompanied by the formation of strongly bound hydroxyl groups. In other words, at the first initial stages of hydration, the water binds strongly to the biopolymer in high energy sorption centers, and in monolayer conformation.¹⁵ As the water starts in multilayer

Fig. 5 **a** Examples of FTIR spectra of films made of PVA-gelatin, conditioned in different salts (represented by numbers in each spectra) and **b** Main changes near 1,730 cm⁻¹ due to moisture content



configuration and free water, with moisture content increasing, no further contributions to the modifications between the polymeric chains take place, probably due to the weaker interactions between the water and biopolymer in this situation. The peaks presented no further variations in their positions. The free water molecules occupy specific interstitial positions between the polymeric chains, favoring their mobility and thus, improving the flexibility, at macroscopic level, without modifying any interactions between the macromolecules, at microscopic scale. In addition, in the films containing glycerol, the changes in the amide II position were found to be less prominent, probably due to the dominance of water in multilayer conformation. Similar changes on the structural and molecular level of gelatin films induced by hydration below 25 % water content (glass–rubbery transition at ambient temperature)

were observed in the literature,¹⁵ and attributed to the way the water is incorporated to the film matrix.

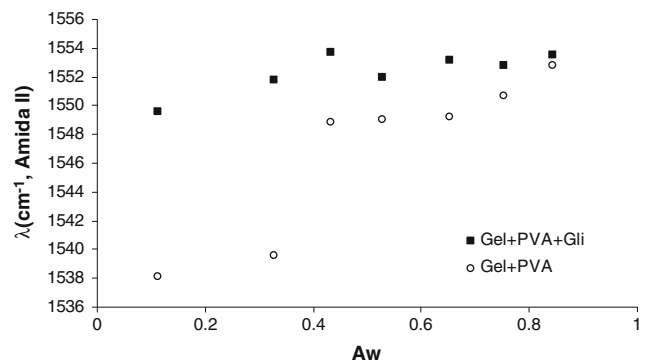


Fig. 6 FTIR peak positions (amide II) of gelatin PVA films with and without glycerol

This behavior can be related by that observed in the dielectric behavior, microwave and gravimetric analysis. In the case of the films without glycerol, the bound water in high energy sorption centers possible contributes in less extent to the dielectric and microwave measurements, due to the presence of strong hydrogen bridge and the high relaxation time of the macromolecules. As the moisture content increases, the dielectric constant also was found to increase, which could be associated to the gradual conversion of water from monolayer to multilayer domain. In the case of films plasticized with glycerol, the lower dependence on the amide II peak with moisture content and the linearity in the microwave response in these films can both be explained on the basis of the predominance of multilayer and free water in the film, which can explain the markedly dielectric response, in relation to that observed in films without glycerol. Finally, the gravimetric results confirm the different way the water enters in the film polymeric matrix.¹⁵

In addition, the acetal groups belonging to PVA was found to be dependent on the water content in all domain of moisture studied, contrarily to that observed in the amide II position. In our parallel works, this phenomenon was observed also in films made only with PVA. This phenomenon could be, therefore, independent on the mechanisms of hydration observed in the gelatin, and, could contribute to the dielectric and microwave response.

To summarize, the different moisture contents on the gelatin films can lead to different effects:

- At lower moisture content, the water molecules occupies high energy sorption centers, via strong hydroxyl bounds, modifying the interactions between the polymeric chains, as seen by FTIR. The strong interactions contributed in less extent to the dielectric and microwave behaviors due to the high relaxation time of the macromolecules.
- The increasing in the moisture content leads to the gradual conversion of water in monolayer, multilayer and free water domains, which do not affect the FTIR peak positions, but due to the high dipolar moment of the water molecule, the dielectric constant and microwave response increased markedly.
- The glycerol affects strongly the moisture content in the edible film, due to its high hygroscopic nature, favoring the multilayer and free water domain, which explains the low FTIR dependence, and higher microwave and dielectric response.
- The changes observed in the remaining acetal groups of PVA due to the water content were observed in all moisture domain studied, and possibly contributes to the dielectric and microwave response of the film.

Conclusions

Therefore, it was possible to get an insight on the way the water molecules interact with the macromolecules, playing important role on structural properties of gelatin films, by means of changes in the polarizability mechanisms and modifications in the strength of the interactions between macromolecules and water, depending on the moisture content, as studied by the methods described in this work. When water molecules are strongly bound to macromolecules, they contribute in low extent to dielectric constant and microwave response and at high extent, to changes in FTIR spectra. At monolayer, multilayer and free water domain, depending on moisture content, the dielectric constant and microwave response increases markedly due to the high polarizability and low relaxation time of water molecules, and the positions of FTIR spectra remain unchanged due to the stabilization of the interactions between bound water already present and the macromolecules.

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Declaration of Conflict Interest The authors declare that they have no conflict of interest.

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