

## On the physico-chemical and dielectric properties of glutaraldehyde crosslinked galactomannan–collagen films

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### Abstract

The effects of galactomannan, a plant polysaccharide widely distributed in nature, on the physicochemical properties of collagen films, were studied using infrared spectroscopy, dielectric spectroscopy, thermal analysis (DSC), swelling and scanning electron microscopy, with possible applications in biomedical, cosmetic and food industry. Infrared bands typical for collagen molecules (amide I, II and III) and galactomannan structure, present in the spectrum of films with 50% of galactomannan, showed that the gum was retained in the collagen fibers. The integrity of the triple helical structure of collagen was conserved in the mixture. The resulting films crosslinked with increasing amounts of glutaraldehyde (GA), when analysed by differential scanning calorimetry (DSC), showed that the reaction induced the presence of two structures, which were the result of the heterogeneous reaction of GA. The presence of galactomannan in the collagen films, increased the swelling, and this effect was inconsistent with GA increments. Scanning electron microscopy showed the entanglement of the galactomannan by fibers of collagen. The piezoelectric strain tensor element  $d_{14}$ , the elastic constant  $s_{55}$ , and the dielectric permittivity  $\epsilon_{11}$  as obtained for the galactomannan–collagen film were studied. Resonance measurement of the piezoelectric strain constant  $d_{14}$  of galactomannan–collagen film (60%) gives 0.081 pC/N.

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### 1. Introduction

Seed gums, like plant galactomannans, are of industrial importance, most notably for use in food, pharmaceuticals, cosmetics, paper products, and paints (Meyer et al., 1993). Galactomannans are plant polysaccharides with a mannan backbone with non-regular substituted (1 → 6) linked  $\alpha$ -D-galactosyl units, which differ in their content of galactose and its distribution along the chain (Dea & Morrison, 1975). The mannose/galactose (M/G) ratio is one of the main chemical characteristics of galactomannans and is dependent on the extraction conditions and the plant source, and determines their physicochemical properties, such as solubility in water, density and the viscosity of solutions. This (M/G) ratio in leguminosus plant varies from 1:1 to 5.7:1 (Scherbukhin & Anulov, 1999). In contrast to mannan, these

polysaccharides are water soluble and form highly viscous solutions. The viscosity appears to be dependent on a number of factors such as molecular weight, degree of substitution, temperature and pH (Wang, Ellis, & Murphy, 2000).

Production and utilization of edible, biodegradable films and coatings prepared from various biological polymers such as polysaccharides, proteins, lipids, or combinations of these components has received great interest in recent years. A limitation in the use of water-soluble galactomannans to produce useful films, is the water sensitivity and brittleness of the cast films. One approach to overcoming these problems could be the preparation of composite films, through combined use of compatible polysaccharides and proteins in order to improve the properties of biopolymers. These composite films can be utilized in the food industry, to graft biosynthesis and in suture and wound dressing production.

Collagen may be an ideal material for these proposed composites since the major applications of collagen-based materials introduced to date include, collagen films, collagen gels and collagen sponges (Piez, 1989).

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Collagen molecules (molecular weight 300,000) are rod-like triple helices, which are 300 nm in length and 1.5 nm in diameter. Collagen fibers possess a high degree of axial alignment of collagen molecules and are characterized by a regular stagger of approximately 1/4 of a rod length between each molecule and its axially aligned neighbour (Nimni, 1988). Soluble collagen can be prepared from tissue, such as skin, by enzyme, acid or alkali treatments. Whereas native collagen tissue possesses significant strength, this strength is lost when collagen products are made from soluble collagen. These reconstituted products may therefore require chemical treatment with crosslinking agents, so as to retain adequate strength for particular applications. Glutaraldehyde is the preferred reagent in the biomedical field and has been used extensively as a crosslinking agent for proteins and polysaccharides. Cross-linking of collagen sample with glutaraldehyde involves the reaction of the free amine groups of lysine or hydroxylysine amino acids residues of the polypeptide chains with the aldehyde groups of the glutaraldehyde. The reaction that will take place is the formation of a Schiff base, thereafter a large variety of subsequent reactions may be involved in the cross-linking material (Cheung, Natasha, Ko, & Nimni, 1985).

Some biopolymers, like collagen and polysaccharides, are found to exhibit the polar uniaxial orientation of molecular dipoles in their structure and can be considered as bioelectrets. Such materials show pyroelectricity and piezoelectricity (Fukada, 1995). Pyro- and piezoelectric studies in various types of biological systems show the presence of natural polarity in the structure of various parts of animals and plants. In many natural structures, polar molecules such as proteins are aligned in parallel with a preferred direction of the polar axis to form crystalline structures. Therefore, such structures can be regarded as natural electrets. Because of this intrinsic polarization, pyroelectricity and piezoelectricity in the axial direction can be observed (Fukada, Ueda, & Rinaldi, 1976). Biocompatible polymeric materials are now used extensively after proper polarization treatment for biomedical applications such as anti-thrombogenic surfaces and artificial membranes (Mascarenhas, 1987).

In this work, we have studied the thermal, dielectric and piezoelectric properties of galactomannan collagen cross-linked films, in order to development of new materials for electronic devices, based on acoustic waves such as surface acoustic wave devices, SAW filter prepared over the surface of a piezoelectric substrates to be used as a pressure sensor in biological applications.

## 2. Materials and methods

### 2.1. Preparation of the soluble collagen

The collagen was prepared by solubilization from bovine serosa after 72 h of treatment under alkaline conditions in

presence of salts, followed by homogenization in acetic acid solution, at pH 3.5 (Goissis & Moriak, 1990). The samples were dialysed against acetic acid solution, and brought to a final concentration of 10 mg/g, determined by hydroxyproline.

### 2.2. Preparation of the galactomannan solution

Galactomannan was obtained by its solubilization from seed endosperms of *Adenanthera pavonina* after homogenization in acetic acid solution at pH 3.5. The solution was centrifuged at 10,000 rpm for 1 h, and the dry matter of the suspension was determined by heating at 100 °C until constant weight. The resultant solution was brought to a final concentration of 10 mg/g.

### 2.3. Preparation of galactomannan–collagen films

The galactomannan–collagen films, were prepared by adding soluble collagen to the galactomannan solution in various proportions ranging from 0 to 70%. The blends were cast in acrylic moulds, and dried in a laminar flow of air.

### 2.4. Crosslinking of films with glutaraldehyde (GA)

For fixation of films with GA, pieces of size 2 cm<sup>2</sup> were immersed in GA solution with variable concentrations from 0.001 to 1.5%, prepared in PB buffer pH 7.4, for 24 h at room temperature. After fixation the pieces were treated in glycine solution (0.025 M glycine:0.05 M borate, pH 9.2) for 10 min, washed exhaustively with water, dried in a laminar flow of air, and used for swelling studies, scanning microscopy and thermal stability assays.

### 2.5. Infrared spectroscopy (IR)

The films for IR analysis were obtained by casting the material in acrylic moulds. A solution of galactomannan 5 mg/g (Gal), a collagen solution at 5 mg/g (Col), and a mixture of the polysaccharide with collagen solution (50% of galactomannan) (GalCol50) was used and dried in dessicator containing P<sub>2</sub>O<sub>5</sub>. Transmission IR spectra were taken in a SHIMATZU FTIR-283B spectrophotometer in the wavenumber region 400–4000 cm<sup>-1</sup>.

### 2.6. Thermal properties

The thermal stability of collagen, galactomannan and galactomannan–collagen films, were determined by DSC, using Shimadzu DSC-50 instrument. Samples were sealed in aluminum cells at a rate of 5 °C/min in a N<sub>2</sub> atmosphere.

### 2.7. Swelling studies

The swelling studies were carried out with pieces of size 1 cm<sup>2</sup> of collagen and galactomannan–collagen films

fixed with variable concentrations of GA (0.001–1%). The films were freeze-dried and immersed in 0.15 M NaCl solution for 24 h at room temperature. The degree of swelling of the samples was calculated using the following equation:

$$\text{Degree of Swelling} = (W_s - W_d)/W_d$$

where  $W_s$  and  $W_d$  are the weights of swollen and dry samples, respectively.

## 2.8. Scanning electron microscopy

The photomicrograph of collagen, and collagen–galactomannan sponges, that were prepared by lyophilization of galactomannan–collagen solutions (with 50, 60 and 70% of the gum), were obtained on a Scanning Electron Microscope, Phillips XL-30, operating at an accelerates voltage in the range of 12–20 keV, for rectangular lyophilized samples, coated with a layer of carbon of 30 nm thickness.

## 2.9. Complex dielectric function measurements

The complex dielectric function measurements were obtained from a HP 4291A material impedance analyzer in conjunction with a HP 4194 impedance analyzer, which jointly covered the region of 100 Hz–1.8 GHz. Fig. 7 shows the sample geometry used for the dielectric and piezoelectric measurements. Rectangular coordinates are assigned to the samples as shown in Fig. 7. The 2–3 plane is the sample plane, and the 1 axis is perpendicular to the plane of the sample. The flat faces of the samples were painted with a silver electrode. The thickness and the diameter of each sample are given in Table 1. For each sample, 100 measurements of the thickness were taken using a digital paquimeter, and used to calculate the average value (see Table 1).

## 2.10. Piezoelectric measurements

The coupled electromechanical equations for our system are:

$$S_i = s_{ij}^E T_j + d_{ij} E_j \quad (1)$$

$$D_i = \epsilon_{ij}^T T_j + d_{ij} T_j \quad (2)$$

$S_j(S_1, \dots, S_6)$  is the strain and  $T_j(T_1, \dots, T_6)$  is the stress,  $E_j$  is the electric field and  $D_i$  is the displacement vector, with constants  $s_{ij}^E$  (compliance for constant electric field),  $\epsilon_{ij}^T$  (dielectric permittivity for constant stress) and the piezoelectric strain element  $d_{ij}$ . Similarly, the coefficients relating the displacement vector ( $D_i$ ) to the strain vector ( $S_j$ ) are called the piezoelectric stress elements  $e_{ij}$ . Piezoelectricity may exist for certain symmetries of crystalline structures. In general anisotropic crystals exhibit the piezoelectric effect and their piezoelectric constants can be represented by the matrix:  $d_{ij}$  (Ikeda, 1996) where the elements of the matrix are called the piezoelectric strain constants. Some of these have null values, according to the symmetry of the material. In the case of natural biopolymers, which show only shear piezoelectricity, the symmetry observed is  $D\infty(\infty 2)$ . In this case,  $d_{25} = -d_{14}$ . In the case of bone, the piezoelectricity appears only when the shearing force acts on the oriented collagen fibers so that they slip past one another (Fukada, 1995). Shear piezoelectricity is observed almost universally for the oriented biopolymers.

Rectangular coordinates are assigned to the film sample. The 1 axis is perpendicular to the plane of the sample, plane 2–3 (Fig. 4).

Using the tensors defined in Eqs. (1) and (2), and taking into account that the electric field is applied in direction 1 ( $E_2 = E_3 = 0$ ), one has:

$$D_1 = \epsilon_{11}^T E_1 + d_{14} T_4 \quad (3)$$

$$S_4 = s_{55}^E T_4 + d_{14} E_1 \quad (4)$$

From Eqs. (3) and (4) one can see the electro-mechanical coupling through the  $d_{14}$  piezoelectric tensor element. In this geometry, one has a mechanical wave propagating in the plane of the sample ( $S_4 \leftrightarrow S_{23}$ ) coupled with the electric field in the ‘1’ axis.

The piezoelectric strain element  $d_{14}$  for the shear piezoelectric is given by Eq. (5):

$$d_{14} = k_{14} \sqrt{\epsilon_{11}^T \epsilon_{55}^E} \quad (5)$$

where:

$$k_{14} = \sqrt{1 - \frac{\epsilon_{11}^S}{\epsilon_{11}^T}} \quad (6)$$

Table 1

Density ( $\rho$ ), thickness ( $e$ ), dielectric permittivity ( $\epsilon$ ), frequency constant ( $fL$ ) and piezoelectricity ( $d_{14}$ )

Sample	$\rho$ (kg/m <sup>3</sup> )	$e$ ( $\mu$ m)	1 MHz $\epsilon_{11}^T/\epsilon_0$	1 GHz $\epsilon_{11}^S/\epsilon_0$	$fL$ (KHz m)	$d_{14}$ (pC N <sup>-1</sup> )
Collagen <sup>a</sup>	1020.62	67.5	3.15	2.66	444.11	0.079
GalCol60–GA01	904.09	51.68	2.70	2.33	406.5	0.081
GalCol70–GA01	1043.55	55.36	2.57	2.18	465.37	0.067

<sup>a</sup> From Góes et al. (2002).

where  $k_{14}$  is the piezoelectric coupling factor,  $\varepsilon_{11}^T$  is the dielectric permittivity obtained by a measurement of the capacitance at a frequency below the fundamental resonance,  $\varepsilon_{11}^S$  is obtained by measurement of the capacitance at a frequency above the resonance mode. The measurement of the dielectric permittivity was made in the range of 1 MHz–1 GHz using the geometry shown in Fig. 7. The values obtained for 1 MHz and 1 GHz can be found in Table 1. In our calculations, we assume  $\varepsilon_{11}^T$  at 1 MHz (below the piezoelectric resonance frequency) and  $\varepsilon_{11}^S$  at 1 GHz (above resonance).

The elastic compliance  $s_{55}^D$ , which is determined from the successive resonance frequencies of the shear mode can be calculated from Eq. (7), assuming that the most pronounced resonances are those where the body can accommodate one half wavelength of the standing elastic wave ( $L = \lambda/2$ ). In this case, one can obtain  $s_{55}^D$ :

$$s_{55}^D = \frac{1}{4\rho(fL)^2} \quad (7)$$

where  $fL$  is the frequency constant which is the product of the resonance frequency and the diameter of the sample (controlling dimension of the sample,  $L \gg e$ ) and  $\rho$  is the sample density. From the experimental measurement of  $\varepsilon_{11}^T$ ,  $\varepsilon_{11}^S$ ,  $fL$  and  $\rho$  one can obtain  $s_{55}^D$  (Eq. (7)),  $k_{14}$  (Eq. (6)) and  $d_{14}$  (Eq. (5)).

### 3. Results and discussion

#### 3.1. Infrared spectroscopy

Fig. 1 shows the IR spectrum of collagen (Col), galactomannan (Gal) and galactomannan–collagen 50% (GalCol50) films. The IR spectra of Col membrane cast at pH 3.5 were characterized by bands at 1650 and 1550  $\text{cm}^{-1}$ , corresponding to the axial deformation of the C=O bond (amide I) and the angular deformation of the N–H bond (amide II) typical of proteins, respectively. The absorption band at 1237  $\text{cm}^{-1}$  is due to the C–N bond in-plane vibration (amide III) and the N–H stretch (amide I), which is related to collagen triple helix structure, and thus sensitive to changes in protein structure. The absorption band at 1450  $\text{cm}^{-1}$  is due to pyrrolidine ring vibrations of proline and hydroxyproline and is not affected by changes in the secondary structure of the protein.

In the IR spectrum of Col there are typical strong absorption bands associated with amide I (C=O) at about 1650  $\text{cm}^{-1}$ , a strong amide II (N–H) at 1550  $\text{cm}^{-1}$  and a band centered at 1237  $\text{cm}^{-1}$ , representing the amide III (C–N) vibrational modes. The ratio between absorption peaks to bands near 1235  $\text{cm}^{-1}$ , which has been shown to be very sensitive to the presence of the tertiary structure of native, and 1450  $\text{cm}^{-1}$  that is related to pyrrolidine ring vibrations showing that the integrity of

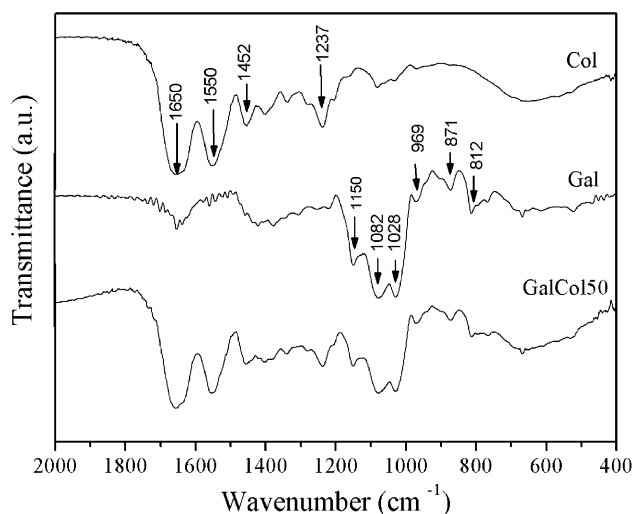


Fig. 1. IR spectrum of collagen (Col), galactomannan (Gal) and galactomannan–collagen GalCol50 films.

collagen structure was maintained (Gordon, Yannas, Buruke, & Lord, 1974).

The IR spectrum of galactomannan film (Gal) shows absorption bands at 812 and 871  $\text{cm}^{-1}$  indicating the presence of  $\alpha$ -linked D-galactopyranose units and  $\beta$ -linked D-mannopyranose units, respectively. Such glycosidic configurations are reported in most seed galactomannan. Other bands are common for polysaccharides, such as values at 1150  $\text{cm}^{-1}$  assigned to bending vibrational modes  $\delta(\text{C–O})$  due to the pyranose ring. The spectral range 1150–950  $\text{cm}^{-1}$  is characterized by the contribution of bending  $\delta(\text{C–OH})$  modes. The band at 978  $\text{cm}^{-1}$  was assigned to deformation of the axial (C–OH) at C-4.

When collagen was mixed with galactomannan in proportions of 50%, (GalCol50), it can be seen that a modification in the spectrum occurs, with bands characteristic of collagen in region at 1650 and 1550  $\text{cm}^{-1}$  of amide II and III, respectively, and bands at 871 and 812  $\text{cm}^{-1}$  suggestive of the presence of the galactomannan once observed in the mixture. The 1235/1450  $\text{cm}^{-1}$  absorption ratios were in the range from 1.0 to 1.1 and are an indication that the collagen triple helix secondary structure was preserved in all cases, since typical values for denatured collagen are close to 0.60.

#### 3.2. Thermal analysis

Fig. 2 shows the DSC thermogram of collagen film (Col), and collagen films crosslinked with GA 0.1% (Col-GA01), for which the denaturation temperatures were 52.2 and 59.3  $^{\circ}\text{C}$ , respectively. DSC thermogram of galactomannan–collagen (50%) films without treatment with GA, show one transition at 50.7  $^{\circ}\text{C}$ . In all galactomannan–collagen 50% films, treated with different concentrations of GA, 0.05 (GalCol50-GA05), 1 (GalCol50-GA1) and 1.5% (GalCol50-GA15), as shown in



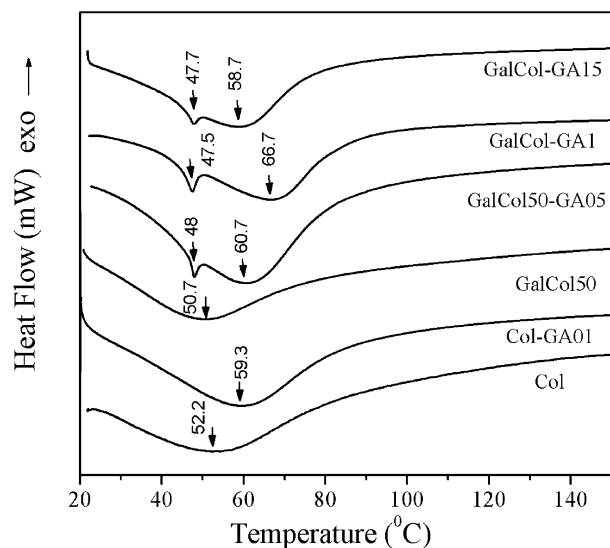


Fig. 2. DSC thermogram of collagen film crosslinked with GA 0 (Col), 0.01% (Col-GA01) and galactomannan–collagen 50% films, treated with different concentrations of GA.

the Fig. 2, the thermograms showed two transitions, the first always near 47 °C and the other between 58 and 67 °C (60.7; 66.7; 58.7 °C for GalCol50-GA05, GalCol50-GA1, and GalCol-GA15, respectively).

Glutaraldehyde stabilizes the collagen molecules and leads to a moderate increase of the denaturation temperature (Casagrande, Wermeister, & Ramshaw, 1994). In the spectrum of both collagen and galactomannan–collagen, without treatment with GA, one can observe a single transition with near thermal stability. The presence of two transitions in galactomannan–collagen films, when cross-linked with different concentration of GA, suggests a mixture of galactomannan and collagen, resulting two structures with different thermal stability. The first transition at low temperature, can be due the collagen not crosslinked with GA. This can be explained by the difficulty of GA access to

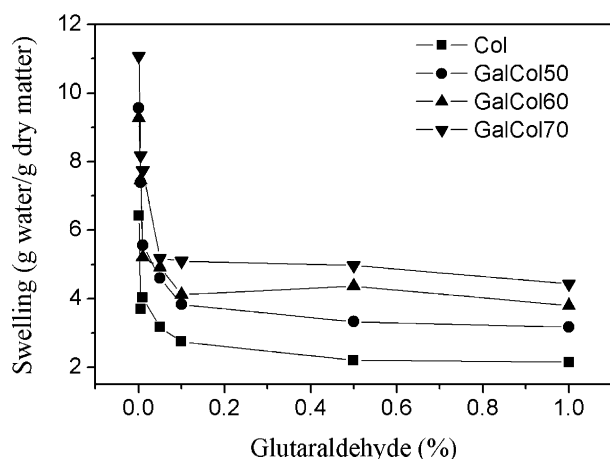


Fig. 3. Rates of swelling of the treated samples as a function of GA concentration (0.001, 0.01, 0.05, 0.1, 0.5 and 1%).

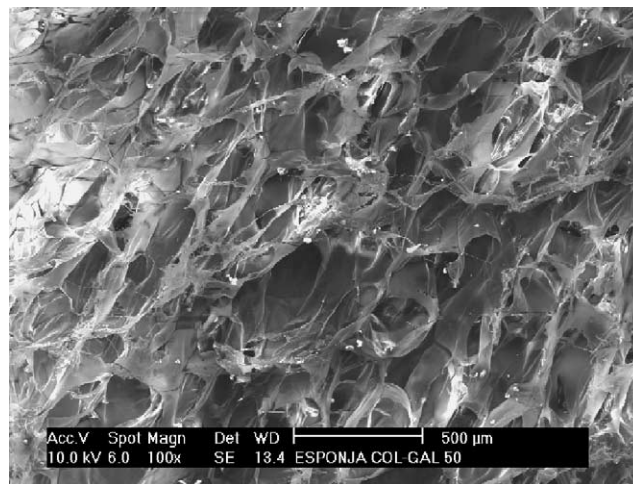


Fig. 4. SEM micrographs of the galactomannan–collagen sponges content 50% of galactomannan.

collagen molecules in the internal matrix of the film, which could be blocked by the presence of galactomannan (Goissis & Figueiró, 1998).

The second transition at higher temperature, 60.7 and 66.7 °C for sample treated with 0.05 and 1% GA, respectively, suggest that the stabilization of collagen molecules exposed at the surface of the film by the action of GA is a function of its concentration. The galactomannan in this structure, can be occluded by the network of collagen fibers resulting the fixation with GA. The transition at 58.7 °C observed for the galactomannan–collagen film treated with 1.5% GA suggests that the occurrence of polymerization reactions of GA at higher concentrations, creating polymers of GA that could also block the penetration of GA monomeric into the internal regions of the matrix of collagen than moving the denaturation temperature to lower values.

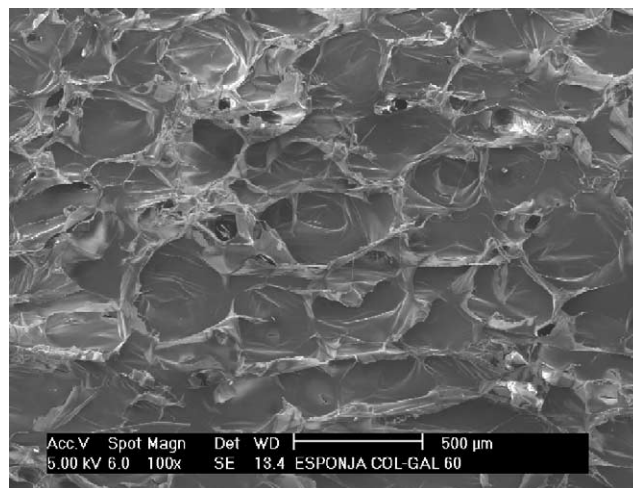


Fig. 5. SEM micrographs of the galactomannan–collagen sponges content 60% of galactomannan.

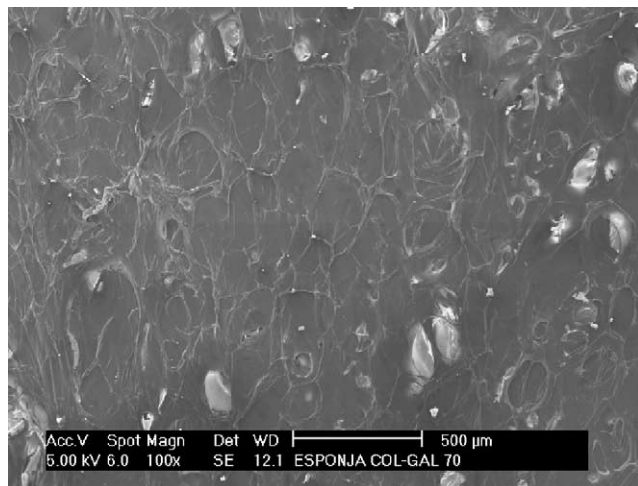


Fig. 6. SEM micrographs of the galactomannan–collagen sponges content 70% of galactomannan.

### 3.3. Swelling studies

The rates of swelling of the treated samples as a function of GA concentration (0.001, 0.01, 0.05, 0.1, 0.5 and 1%) are presented in Fig. 3. The degree of swelling for all samples decrease as a function of the GA crosslinking, and reached an equilibrium state at 0.1% GA. There is a significant change in swelling that results from an increase of galactomannan concentration in the films, with a values ranging from 6.42 to 11.08 g water/g dry matter to 0 and 70% of galactomannan concentration at 0.001% GA crosslinking. These results suggest that the GA crosslinking of collagen molecules in the blended networks promotes a reduction in the swelling of the samples.

## 4. Scanning electron microscopy

SEM micrographs of the galactomannan–collagen sponges (50, 60 and 70% of galactomannan) are shown in Figs. 4–6. SEM images provided insight into the structural features of the surfaces of the sponges. At 50% galactomannan content (Fig. 4) one can see the characteristic bundles of collagen fibres that entangle the galactomannan

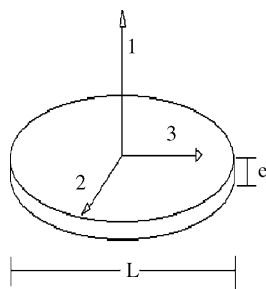


Fig. 7. A rectangular coordinate system assigned to the sample for the measurement of piezoelectric and dielectric permittivity.

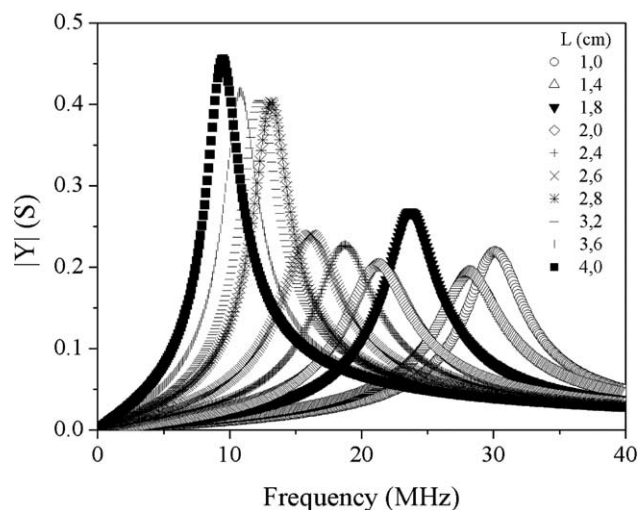


Fig. 8. Frequency dependence of the absolute value of the admittance,  $|Y|$ , for galactomannan–collagen film treated with GA 0.1%, sample GalCol60-GA01 for different electrodes size.

component, which appear as a smooth surface in the sponge. The SEM micrographs of galactomannan–collagen sponges containing, 60 and 70% of the gum (Figs. 5 and 6, respectively), show an increasing smoothness of the surface as the galactomannan content increases, while the collagen fibres become hidden on the surface of sponges.

## 5. Piezoelectricity

The thickness and the diameter of each sample are given in Table 1. For all the studied samples the diameter  $L$  varied between 1 and 4 cm. Measurement was made with the sample in disk type geometry (Fig. 7). Figs. 8 and 9 show the frequency dependence of the absolute value of

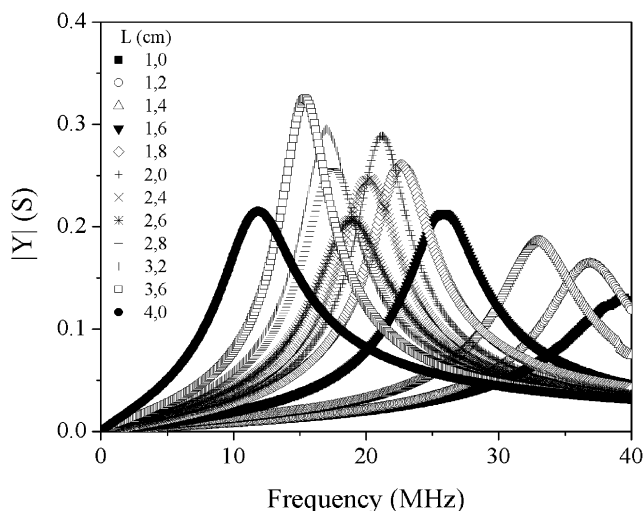


Fig. 9. Frequency dependence of the absolute value of the admittance,  $|Y|$ , for galactomannan–collagen film treated with GA 0.1%, sample GalCol70-GA01 for different electrodes size.

Table 2  
Loss factor  $Q^{-1}$

Sample	L (cm)											
	1	1.2	1.4	1.6	1.8	2.0	2.4	2.6	2.8	3.2	3.6	4.0
GalCol60-GA01	0.13	–	0.17	–	0.15	0.22	0.23	0.25	0.18	0.21	0.22	0.24
GalCol70-GA01	–	0.16	0.15	0.20	0.20	0.17	0.22	0.28	0.25	0.22	0.21	0.46

the admittance,  $|Y|$ , for different diameters of the electrode disk, to galactomannan–collagen films treated with GA 0.1%, samples GalCol60-GA01 and GalCol70-GA01, for films with 60 and 70% of galactomannan, respectively. From Figs. 8 and 9 one can confirm the reduction of the resonance frequency associated with the increase of the electrode diameter ( $L$ ). This is an expected result associated with the main characteristic of the acoustic resonator. This resonance is directly associated to the piezoelectric resonance of the material. The electromechanical constants of a piezoelectric material are determined by the admittance measurement of a transducer with varying frequency. For practical purposes it is sufficient to observe the admittance around its fundamental frequency. From these figures, the frequency constant,  $fL$  associated with the shear piezoelectric mode of this sample was obtained. With the experimental measurements of  $\varepsilon_{11}$ ,  $fL$  and  $\rho$  and using Eqs. ((1)–(3)) one can obtain the piezoelectric strain element  $d_{14}$  for the shear piezoelectricity (see Table 1). The piezoelectric strain tensor element  $d_{14}$  obtained for collagen was around 0.079 pC/N (Góes, Figueiró, Paiva, Vasconcelos, & Sombra, 2002). For sample GalCol60-GA01 the piezoelectric tensor element was 0.081 and for GalCol70-GA01 it was 0.067. The decrease of the value of the piezoelectricity observed for GalCol70-GA01 suggests the low stability of films with high galactomannan content. The loss factor  $Q^{-1}$  of the samples was also measured using the admittance resonance method (see Table 2). On average for the higher electrodes diameters the film GalCol60-GA01 showed lower values when compared with the sample GalCol70-GA01.

## 6. Conclusions

The IR results suggest that the triple helix structure of collagen was not affected with the presence of galactomannan. This was by the presence of typical bands in the spectrum for both collagen and the galactomannan, indicating that the polysaccharide was also retained in the film. However, for galactomannan–collagen films (50%), the spectrum did not show any shift of characteristics bands of collagen or galactomannan, which suggest that no interaction or binding occur between these molecules. Results obtained from thermal analysis (DSC) of galactomannan–collagen films crosslinked with GA,

show the presence of two structures, which suggest that GA reaction is not homogeneous. The GA treatment was found to decrease the swelling in galactomannan–collagen films and this also occur as a function of galactomannan concentration in the films. The scanning electron micrographs showed the entanglement of the galactomannan by the collagen fibers. The piezoelectric strain tensor element  $d_{14}$ , the elastic constant  $s_{55}$ , and the dielectric permittivity  $\varepsilon_{11}$  were obtained for the galactomannan–collagen film. Resonance measurement of the piezoelectric strain constant  $d_{14}$  of galactomannan–collagen (60%) film gives 0.081 pC/N.

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