RHEOLOGICAL CHARACTERIZATION OF SOLUTIONS OF GELATIN WITH BENTONITE AND TANNIC ACID

CARACTERIZACIÓN REOLÓGICA DE SOLUCIONES DE GELATINA CON BENTONITA Y ÁCIDO TÁNICO

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Abstract

This work aimed to study the effect of bentonite particle size, on the rheological properties of gelatin solutions with glycerol, bentonite, and tannic acid. To decrease the particle size, a bentonite solution was centrifuged, recovering only the supernatant (treated bentonite), both control and treated bentonite solutions (0.025 g bentonite / g water) were characterized using atomic force and confocal microscopy. Six solutions, named as C (100, 25, 0, 0, 0), TA (100, 25, 8.47, 0, 0), B (100, 25, 0, 16.83, 0), TB (100, 25, 0, 0, 16.83), O (100, 25, 8.47, 16.83, 0) and TO (100, 25, 8.47, 0, 16.83) g / 4500 g water, prepared with gelatin, glycerol, tannic acid, control bentonite, and treated bentonite respectively, were subjected to rheological tests (flow curves and viscoelasticity). The treatment of bentonite reduced the particle size about 200 nm. The solutions O and TO showed shear-thinning behavior (n < 1) with higher moduli (G' and G") values, than C, TA, B and TB at both 10 and 25 °C. The solutions with treated bentonite produced an improved gel structure, showing less temperature dependence

Keywords: montmorillonite, non-Newtonian behavior, viscoelasticity, particle size, gelatin solutions.

Resumen

Este trabajo tuvo como objetivo estudiar el efecto del tamaño de partícula de la bentonita, en las propiedades reológicas de las soluciones de gelatina con glicerol, bentonita y ácido tánico. Para reducir el tamaño de partícula, se centrifugó una solución de bentonita, recuperando sólo el sobrenadante (bentonita tratada). Las soluciones de bentonita control y bentonita tratada (0.025g de bentonita / g de agua), se caracterizaron utilizando microscopía de fuerza atómica y confocal. Seis soluciones, nombradas como C (100, 25, 0, 0, 0), TA (100, 25, 8.47, 0, 0), B (100, 25, 0, 16.83, 0), TB (100, 25, 0, 0, 16.83), O (100, 25, 8.47, 16.83, 0) y TO (100, 25, 8.47, 0, 16.83) g / 4500g de agua, de gelatina, glicerol, ácido tánico, bentonita control y bentonita tratada respectivamente, fueron sometidas a ensayos reológicos (curvas de flujo y viscoelasticidad). El tratamiento de la bentonita redujo el tamaño de partícula en aproximadamente 200 nm. La soluciones de O y TO mostraron un comportamiento adelgazante (n <1) y con valores superiores de los módulos (G' y G"), en comparación a las otras soluciones, a 10 y 25 °C. Las soluciones con bentonita tratada produjeron una estructura de gel reforzada, mostrando una menor dependencia de la temperatura.

Palabras clave: montmorillonita, comportamiento no Newtoniano, viscoelasticidad, tamaño de partícula, soluciones de gelatina.

1 Introduction

In the last years, new materials have been developed from biomolecules and cross-linking agents (Cao *et al*., 2007; Herrera-Franco *et al*., 2016), along with nanometric sized inorganic solids (Jorge *et al*., 2014; Jorge *et al*., 2015) These nanostructured macromolecules or nanocomposites, have outstanding properties for the food packing industry, which may be regulated by choosing suitable components, as well as by controlling the mixture preparation process (Qazvini *et al*., 2012; Ramírez-Hernández *et al*., 2015).

Gelatin is one of the biopolymers used for creating nanocomposites for its application in the food

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packaging industry. Besides, its study, improvement and use, are still broadly investigated (Mhd-Sarbon *et al*., 2013; Jorge *et al*., 2014; Andrade *et al*., 2015; Jorge *et al*., 2015). Thus, the searching of its reinforcement by the addition of restructuring and strengthening materials, is of great importance.

Laminated silicates are widely used as strengthening materials, due to its capacity to improve biopolymer functional properties, preserving its biocompatibility (Qazvini *et al*., 2012). Montmorillonite is one of the commonly used clays; it consists of one nm thick aluminum silicate layers, with a negative charge on its surface, which is balanced by ions intercalated between such layers. These ions may be Ca^+, Mg^+ or Na^+. When Na^+ is the only one intercalated in the overall structure, it is known as Na+-montmorillonite, also named as Bentonite from (Luckham and Rossi, 1999).

Bentonite supplied either orally or topically has been used as an antacid, gastrointestinal protector, antidiarrheal, as a dermatological protector in cosmetic creams, powders, and emulsions (Carretero and Pozo, 2009). Bentonite does not cause toxicity when ingested or when injected into subcutaneous tissues of mice, but it provokes euthanasia when injected intraperitoneally or intravenously at non-fatal doses under five milligrams (Hudson *et al*., 2008). In films from gelatin with chitosan and Na+-montmorillonite, a high biocompatibility with no toxicity for cell growth at lower concentrations than 18% (w/w) has been observed (Zhuang *et al*., 2007). Besides, since bentonite is not absorbed in the human gut, probably there is no way it can be potentially toxic when in contact with foods in small amounts.

Talibudeen (1955) and Panzavolta *et al*. (2014), have studied the formation of the compound bentonite-gelatin, which is produced by the interchange of Na^+ ions with the NH_3^+ groups on the lateral chains, from the amino acids proline and hydroxyproline in the gelatin structure, giving as result ionic interactions. The viscoelastic properties of gelatin solutions with bentonite have been studied, observing an increment in the transition sol-gel, with interactions depending to a great extent on the pH value (Qazvini *et al*., 2012; Jorge *et al*., 2014).

The use of crosslinkers in gelatin is an effective way to create stable covalent bonds among its polypeptides; this gives as a consequence an improvement in the mechanical properties and water resistance of gelatin packing materials (Zhang *et al*., 2010a). Compounds like glutaraldehyde, genipin and phenolic compounds have been used as crosslinkers,

among which tannic acid can be mentioned as an example (Van Buren and Robinson, 1969; Bigi *et al*., 2002; Cao *et al*., 2007; Zhang *et al*., 2010a).

The tannic acid (β-1,2,3,4,6-Pentagaloil-O-D-glucose) is a polyphenolic compound, consisting of a glucose nucleus, linked to five gallic acid molecules by ester bonds (Hagerman *et al*., 1992). Previous studies have found that tannic acid can interact with gelatin in two ways. The first one is under alkaline conditions; its polyphenolic sites can react with basic amino acids from gelatin (lysine, arginine and histidine) forming C-N covalent bonds, giving place to cross-links networks (Zhang *et al*., 2010b). The second way is by creating hydrogen bonds between hydroxyl groups of tannic acid, and carbonyl groups of gelatin (Van Buren and Robinson, 1969).

It is well known that the rheological properties are important in process design and modeling, and these properties are taken in some cases as an indicator of packing products quality. The viscoelastic properties of gelatin solutions furnish information on its molecular arrangement (Karazhiyan *et al*., 2009; Chandra and Shamasundar, 2015). Besides, this type of tests shows the influence of various parameters on the stability of its triple helix formation, since such helices form networks that strongly modify the viscoelastic properties of gelatin solutions (Joly-Duhamel *et al*., 2002).

At present, there are not previous reports in the literature about the rheological properties of gelatin solutions with bentonite and tannic acid, used as strengthening or restructuring agents respectively. Besides, no studies focused on the effect of bentonite particle size in gelatin solutions have been found. That is why the aim of this work was to study the effect of bentonite particle size in the rheological properties of gelatin solutions with glycerol, bentonite (control and treated) and tannic acid.

2 Material and methods

2.1 Materials

For the elaboration of bentonite solutions, hydrophilic montmorillonite (Nanomer® PGV, Sigma-Aldrich, USA) was used. For film manufacture, technical grade bovine gelatin (type A, 275 °Bloom, Duche, Mexico), analytical grade glycerol (Fermont, Mexico), and analytical grade tannic acid (gallotannic acid, JT Baker, USA) were used.

2.2 Preparation of bentonite solutions

Two kinds of bentonite solutions were used independently, i.e., control and treated bentonite. To prepare control bentonite solution, 25 g bentonite were dispersed in 1000 g of deionized water and hydrated for 12 hours. After that, this solution was homogenized at 4500 x g with a mixer (ULTRA-TURRAX® IKA, T25 digital, Germany) during 10 min, and then, sonicated in an ultrasonic bath (Sonicator Branson, 2510, USA) at 50° C and 40 KHz, for 30 min.

To decrease the particle size of bentonite, the modified method of Cadene *et al.* (2005) was used. About 200 g of bentonite were diluted in 1000 g of deionized water; this was homogenized at 6000 x g with the above mentioned mixer for 30 min, and then, it was centrifuged at 10621 x g (Centrifuge Beckman, Avanti 30, USA) for 15 min. The supernatant obtained was subjected again to the same centrifugation process, repeating this cycle a total of three times. Then, the dry weight of bentonite solutions (control and treated) was obtained and adjusted to a concentration of 0.025 g bentonite / g water.

2.3 Preparation of bentonite solutions

2.3.1 Particle size and Zeta potential

The determination of the average particle size of both control and treated bentonite solutions, were made by photon correlation spectroscopy, with a ZetaPlus spectrometer (Brookhaven Instruments Company, USA). The Zeta potential of the solution samples was obtained using the same equipment, by measuring the electrophoretic mobility at 25 °C.

2.3.2 Atomic force microscopy

To observe the morphology and particle size of bentonite, an NT-MDT atomic force microscope (NT-MDT, Solver Next, Russia) in tapping mode was used. About one μL of bentonite solution was dissolved in 150 μL of milliQ water, an aliquot of 10 μL was taken and dropped over a mica plate, which was then dried at room temperature for 24 h before the test.

For determination of the dimensions of clay particles, AFM images were digitalized using Nova Px image analysis software (Nova Px 3.1.0.0 Image Analysis Software NT-MDT, 2007), the major and minor length axes were determined directly from the images, and the data were analyzed with the program Sigma Plot version 11.0 (Systat Software, 2008).

2.3.3 Laser scanning confocal microscopy

Laser scanning confocal microscopy (LSM 710, Carl Zeiss, Germany), was also used to observe the bentonite morphology. About 1 mL of bentonite solution was dropped over a mica plate, then, the laser was adjusted to green / red fluorescence at a wavelength of 450 nm. Images (1138 x 1138 pixels) were obtained with a magnification of 100X with an Epiplan-Neofluar (50x/1.30 oil-pool objective). Using image analysis techniques; the lacunarity, were characterized quantitatively. The processing was done using the software ImageJ version 1.39u (National 193 Institute of Health, 2008). This consisted in making the images binary, then, parameters were evaluated with Frac Lag 2.5, and the lacunarity data were analyzed with the program Sigma Plot version 11.0 (Systat Software, 2008).

2.4 Preparation of gelatin solutions

Six solutions were prepared according to Table 1, using the modified method of Cao *et al.* (2007). The concentrations were decided on basis to a previous study (Ortiz-Zarama *et al.*, 2016), with constant concentrations of gelatin (100 / 4500 g water) and glycerol (25/ 100 g gelatin). The gelatin was hydrated in distilled water at room temperature during 12 h, then, it was subjected to constant agitation using a magnetic stirrer for 30 min at 50 °C. For TA, O and TO solutions, the tannic acid was solubilized in 100 g of deionized water and added to the gelatin solution, and for C, B and TB solutions only deionized water was added to the gelatin solution. The pH of solutions of gelatin, or gelatin/tannic acid, was adjusted to about 9 with a 1 M KOH solution and maintained with constant agitation for 60 min at 50 °C to favor reticulation (crosslinking formation) reactions.

For the solutions with treated and control bentonite (O, TB, B and TB), glycerol was added to bentonite solution (the concentration was adjusted according to Table 1), this was then let in rest for 16 hours before its homogenization at 4500 x g for 10 min and subsequent sonication in the mentioned ultrasonic bath at 50° C and 40 KHz for 30 min. The solution bentonite/glycerol was added to the either gelatin or gelatin / tannic acid solution slowly (drop by drop), at 50 °C during 6 hours. For the solutions C and TA, glycerol was dissolved in 100 g of water and

Table 1: Nomenclature and concentrations of control and treated bentonite, and tannic acid used to obtain gelatin solutions. The gelatin and glycerol concentrations were constant (100 g 4500 g^{-1} water and 25 g 100 g^{-1}gelatin respectively).

Solution name	Tannic acid	Control bentonite	Treated bentonite
		(g 100 g^{-1}gelatin)	
O	8.47	16.83	-
TA	8.47	-	-
B	-	16.83	-
C	-	-	-
TO	8.47	-	16.83
TB	-	-	16.83

this was added to the either gelatin or gelatin / tannic acid solution, once the addition finished, the resulting composite solution was homogenized (4500 x g) for 10 min and then sonicated at 50° C and 40 KHz, for 30 min.

2.5 Rheology of gelatin solutions

Rheological tests were done with a TA Instruments stress controlled rheometer (AR1000, USA), using a cone and plate geometry (cone angle of 2°, 60 mm diameter) assembled with a Peltier system to control the temperature, all tests were done on different samples (triplicate). To run flow curves, about 2 mL of sample were placed onto the equipment fix plate; this test was done at 25 °C, where the samples were in a sol state.

The solutions viscosity was calculated using the Newtonian model (Ec. 1), as the shear stress (τ, Pa), divided by the shear rate (γ, s^{-1}) within the range of 0 to 300 s^{-1}. When the determination coefficient (R^2) was in the order of 0.95, the behavior of the solutions was described by the power law model (Ec. 2), where k is the consistency coefficient (Pa.s^n), n is the flow behavior index (dimensionless) (Tadros, 2011).

$$\mu = \tau/\gamma \qquad (1)$$
$$\tau = k\gamma^n \qquad (2)$$

The viscoelasticity of the solution samples was evaluated by undertaking dynamic or oscillatory tests, running amplitude stress sweeps from 0.1 to 1 Pa at a constant frequency of 1 Hz, to find the linear viscoelastic region (LVR), both in gel (at 10 °C) and sol state (at 25 °C). The frequency sweeps were run from 0.1 to 10 Hz, with a stress of 0.63 Pa both at 10 °C and at 25 °C. All oscillatory tests were done isothermally. The storage or elastic modulus (G') the loss or viscous modulus (G") and the Tan(delta) or loss tangent (G"/G') were evaluated using the software from the equipment.

To observe possible changes in the rheology of gelatin solutions in function of temperature, temperature sweeps were run by cooling down samples from 25 to 10 °C at a heating rate of 1 °C / min, and constant values of frequency (1 Hz) and stress amplitude (0.63 Pa). The gel point (Tsol-gel), was evaluated as the temperature where the plots of both moduli (G' and G") displayed a cross-over.

2.6 Statistical data analysis

To identify differences in particle size, Zeta potential, major length axe, minor length axe and lacunarity for the treatments of bentonite; an analysis of variance (ANOVA) was applied using the program SigmaPlot version 11.0 (Systat Software, 2008). Also, for gelatin solutions, the viscosity, loss tangent, relaxation time and the gel point (Tsol-gel) were analyzed, followed by the Tukey multiple comparisons procedure when differences were found. The significance level (P) was 0.01 in all cases.

3 Results and discussion

3.1 Characterization of bentonite solutions

3.1.1 Particle size and Zeta potential of control and treated bentonite solutions

It was observed a decrease of about 200 nm on the bentonite particle size in the aqueous solution, as an effect of the undertaken treatment, showing a minimum size of about 450 nm (Table 2). The decrease in particle size also reduced the Zeta potential value. It is known that the magnitude of such Zeta potential is an indication of the potential stability of colloidal systems. All particles in suspension with a Zeta potential higher than +30 or lower than -30 mV, tend to repel each other, being considered stable. But if its Zeta potential lies between +30 to -30mV, they tend to attract each other and to flocculate (Tunç and Duman, 2010).

Table 2: Particle size, Zeta potential, major length, minor length and lacunarity from control and treated bentonite solutions*.

	Particle size (nm)	Zeta potential (mV)	Major length axe (nm)	Minor length axe (nm)	Lacunarity
Control bentonite	624.89 ± 35.43^a	-33.04 ± 3.61^a	0.71 ± 0.15^a	0.48 ± 0.09^a	0.59 ± 0.05^a
Treated bentonite	450.53 ± 29.95^b	-44.62 ± 3.61^b	0.49 ± 0.19^b	0.37 ± 0.07^b	0.47 ± 0.03^b

*Each value is the mean of three determinations ± standard deviation, different letters within each column presented significant differences ($P < 0.05$)

Thus, the Zeta potential values found in this work for the control bentonite solution (approximately -33mV), suggests that bentonite dispersion is in the limit of stability. However, the treated bentonite showed lower values than the limit of -30mV, thus, its aqueous solution was much more stable. Mekhamer (2010) found Zeta potential values for bentonite solutions between -30 and -40 mV when they were sonicated for 120 min.

3.1.2 Atomic force microscopy

Fig. 1 shows images of the bentonite sizes seen by atomic force microscopy, differences in sizes are observed between control (major and minor length axes were 710 and 480 nm respectively) and treated (major and minor length axes were 490 and 370 nm respectively) samples. Nonetheless, it is worth noting that although these values are near as those reported in particle size (Table 2), the trend is the same (control > treated). Besides, it is important to emphasize that the measurements were undertaken after drying the samples for 24 h, and under such conditions the bentonite may tend to agglomerate, giving as result larger particles (Mingelgrin *et al.*, 1978).
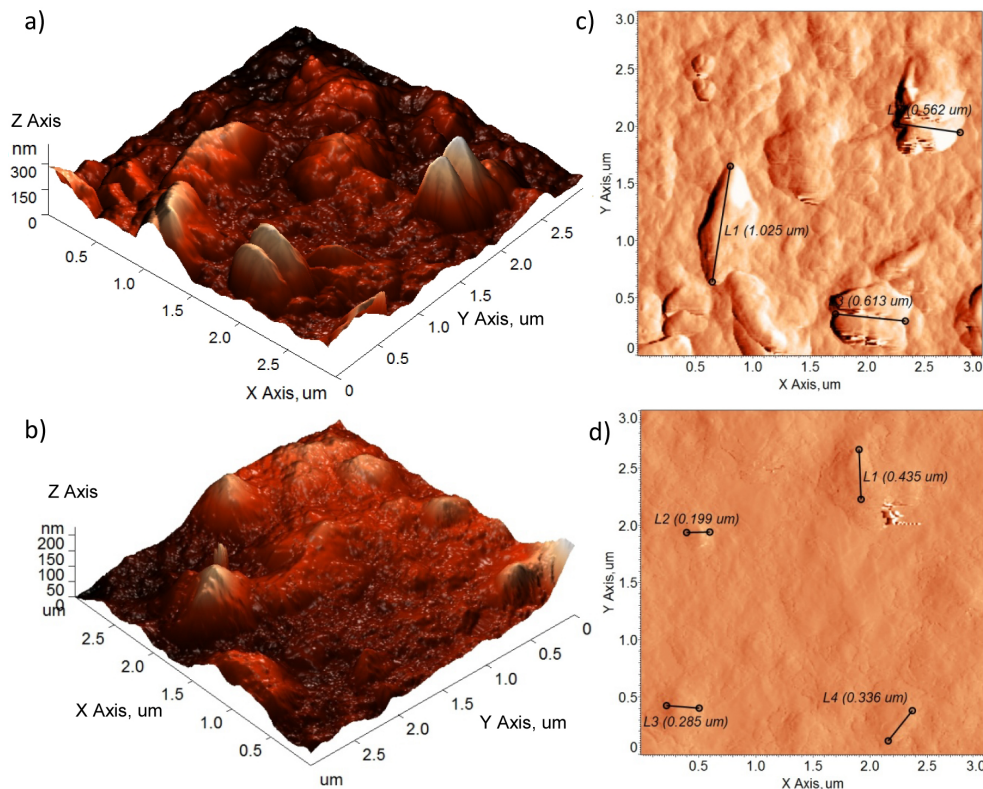

Fig. 1: Micrographs of height (3D) and amplitude (2D) from control (a and c) and treated bentonite (b and d); obtained by atomic force microscopy
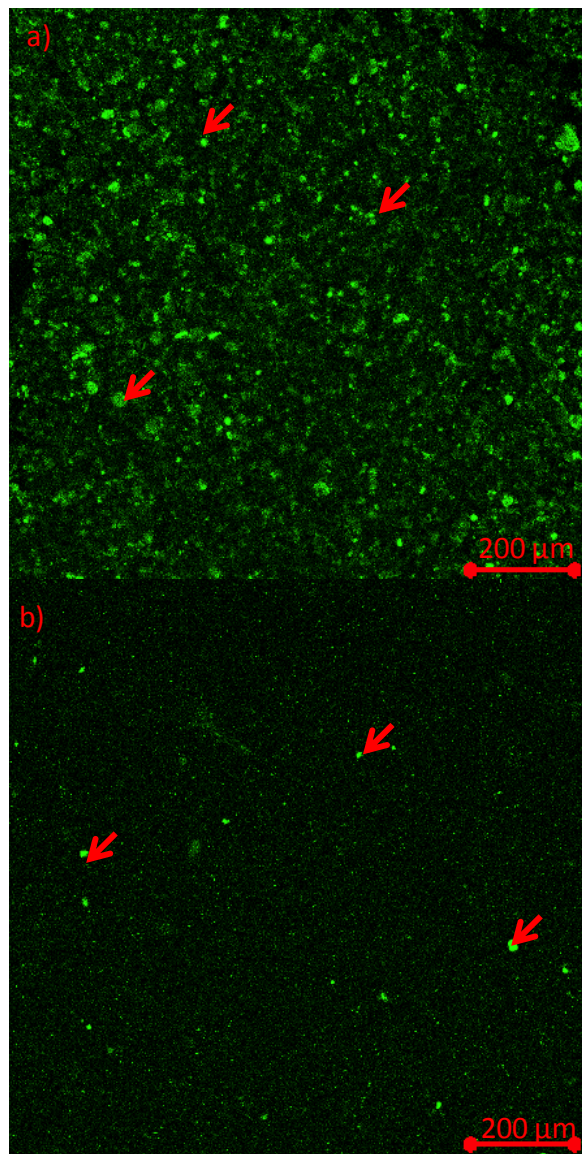
Fig. 2: Laser scanning confocal microscopy of dispersion samples from a) control and b) treated bentonite, all with 0.025 g bentonite/ g water. The red arrows show bentonite aggregates.

For the control bentonite (Fig. 1 a and b), it was found that there is not a consistent particle size, with changes in shape, but the aggregates show borders in sort of layers, supporting the theory of the crystalline growth (Cadene *et al.*, 2005). However, for the treated bentonite (Fig. 1 c and d) particles with more regular shape and size are observed. Cadene *et al.* (2005) found a wide variety of forms with a significant dispersion in length, showing an average value of 350 nm when studying the natural Na$^+$-montmorillonite.

3.1.3 Laser scanning confocal microscopy

The green points observed in the micrographs (Fig. 2), represent the crystalline bentonite with its intrinsic photoluminescence at 550 nm. The control bentonite (Fig. 2a) image shows a lot of aggregates (red arrows), these have different sizes and shapes, a similar scenario to what was seen in the AFM micrographs described previously. Respect to the treated bentonite (Fig. 2b), a lower number of aggregates with smaller sizes (maximum size of 10μm, with an average of 400nm), and the particle sizes and shapes are more homogeneous than those of the control specimen. The lacunarity values found (Table 2) shows a better distribution of cloisite in treated bentonite (i.e., a decrease 0.59 to 0.47) than control bentonite.

Liu *et al.* (2011) in a previous study on nanoclays (cloisite 20A), obtained similar micrographs when the effect of its addition in poly(styrene-co-acrylonitrile) nanocomposites were analyzed, finding that the less the nanoclay concentration, better its dispersion, with similar images to those found for the treated bentonite from this work. However, the effect observed in this work was probably due mainly to the bentonite treatment, since the concentrations of both control and treated nanoclay were the same (0.025 g bentonite / g water).

3.2 Rheology of gelatin solutions

3.2.1 Rheological profiles

For the solution samples C, TA, B and TB (Table. 1), Newtonian behavior ($R^2 > 0.995$) was found (Fig. 3), with viscosity values of $4.79 \times 10^{-3} \pm 6.6 \times 10^{-5}$, $5.79 \times 10^{-3} \pm 9.2 \times 10^{-5}$, $8.2 \times 10^{-3} \pm 6.1 \pm 10^{-4}$ and $2.11 \times 10^{-2} \pm 2.23 \times 10^{-3}$ Pa.s, respectively, with a statistically significant difference (P ≤0.05) among all samples. Bentonite and tannic acid increased the solutions viscosity; however, the higher increase in viscosity was that observed in sample TB, being four times as much as that from C, which in turn, twice as much as that was shown in B. This was mainly due to the higher superficial area displayed by the treated bentonite, which made it capable of forming a higher number of hydrogen bonds, giving as result an increase in viscosity (Jorge *et al.*, 2014). On application of the power law model to the solutions O and TO (Table. 1) the following Ec. (3)-(4) were obtained:

$$O : \tau = 0.0627\gamma^{0.908} \quad R^2 = 0.999 \quad (3)$$
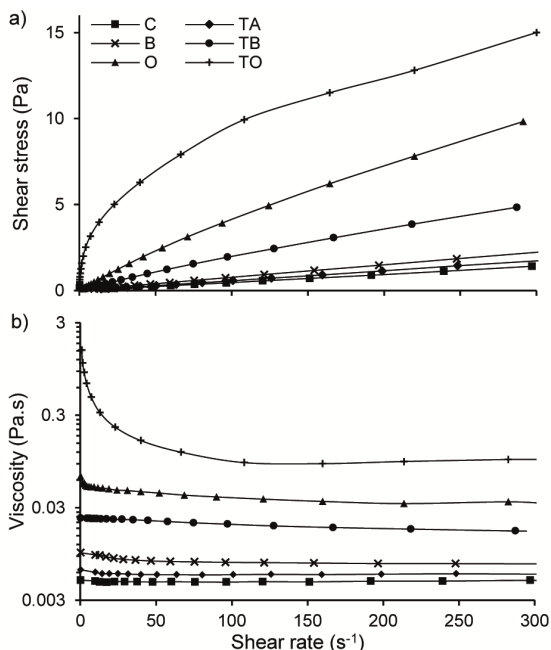$$TO : \tau = 1.0532\gamma^{0.498} \quad R^2 = 0.977 \quad (4)$$

Fig. 3. Rheological profiles from gelatin solutions. C(100, 25, 0, 0,0), TA(100, 25, 8.47, 0, 0), B(100, 25, 0, 16.83, 0), TB(100, 25, 0, 0, 16.83), O(100, 25, 8.47, 16.83, 0) and TO(100, 25, 8.47, 0, 16.83) g / 4500 g water, prepared with gelatin, glycerol, tannic acid, control and treated bentonite respectively. a) flow curves and b) viscosity curves (0-300s^{-1}). All at 25 °C.

Both O and TO solutions had a shear-thinning behavior (n < 1), i.e., there was a decrease in viscosity as the shear rate increased, this was due to the gradual microstructure breakage in the gelatin solutions when the applied shear rate value was rising. Within the involved shear rates, the following decreasing order in viscosity values was found; TO > O > TB > B > TA > C, as seen in Fig. 3b. Similar results were observed by Jorge *et al*. (2014) working on gelatin-montmorollonite solutions.

The increase in viscosity is the result of a higher restriction of the molecular movement, due to higher cross-links among the polymer chains (Chandra and Shamasundar, 2015). Strengthening of the interactions of all compounds in formulations O and TO gave an increase in viscosity and in sample TO this was enhanced by the effect of the treated bentonite.

Leuenberger (1991), reported that usually gelatin solutions have a Newtonian behavior, being affected by the gelatin concentration, the temperature or the gelatin molecular weight. It has been found that glycerol decreases the viscosity of gelatin solutions

(Andrade *et al*., 2015), which is probably why lower viscosity values were found for solution C. But, it might be affirmed that the bentonite effect (increase in viscosity) predominated over that of glycerol.

3.2.2 Dynamic tests

Fig. 4a shows results of amplitude tests for all gelatin solutions, undertaken over the range 0.1 to 1 Pa at 10 °C (below the sol-gel transition temperature). All plots are overall flat, with no real breaking points which could be an indication of structure disruption. In all cases, both the storage and the loss moduli (G', G") were independent of the applied stress at a frequency of 1 Hz (linear viscoelastic region), and it is also seen that G' is higher than G". From these profiles, it was decided to choose 0.63 Pa which is within the linear domain, to run all frequency sweeps.

The frequency profiles obtained at 10 °C (Fig. 4b) shows that G' is much higher (about ten times as much) than G" for all formulations over the frequency range involved, which is typical of "strong" gels (Mhd-Sarbon *et al*., 2013). The values of both moduli (G' and G") were frequency-dependent at low frequencies (from 0.1 to 1 Hz), where all solution samples showed values of G' with a trend to increase, but from 1 Hz onwards, this modulus showed a linear behavior.

The behavior of G" (Fig. 4b) at 10 °C was different, on the one hand, from 0.1 till about 2.1 Hz its values increased in samples TO, TA and O, that is to say in formulations that have tannic acid. On the other hand, for the formulations without tannic acid (TB, B and C), G" decreased within the mentioned frequency range. In all cases, for the frequency range from 2.1 to 10 Hz, the trend of G" was to be frequency independent.

Joly-Duhamel *et al*. (2002), found a strong correlation between the storage modulus and the number of triple helices; the larger the G' value, the greater the number of triple helices. They also proposed that the loss modulus reflects the presence of dangling ends, loops attached to the network, or free chains, which contribute to the dissipation of energy by friction but not to the elastic network strength. So it can be inferred that the tannic acid contributes to the flexibility of the free chains of gelatin (long flexible coils), while bentonite decreases the flexibility of these joints, and both tannic acid and bentonite, diminish the length of triple helices.
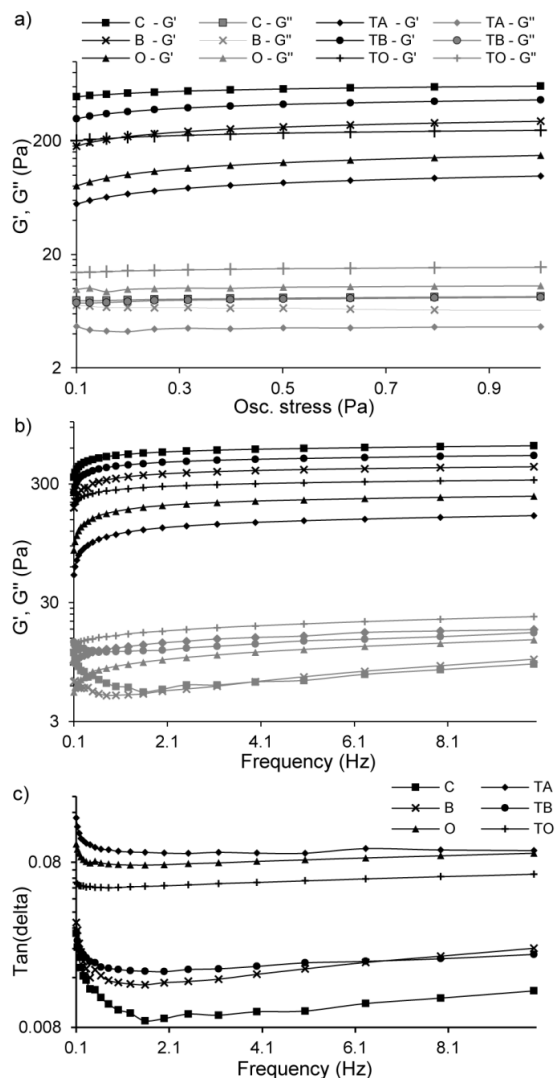
Fig. 4. Rheological profiles from gelatin solutions. C (100, 25, 0, 0,0), TA (100, 25, 8.47, 0, 0), B (100, 25, 0, 16.83, 0), TB (100, 25, 0, 0, 16.83), O (100, 25, 8.47, 16.83, 0) and TO (100, 25, 8.47, 0, 16.83) g / 4500 g water, prepared with gelatin, glycerol, tannic acid, control and treated bentonite respectively. a) Amplitude sweep (G', G''), b) frequency sweep (G', G''), and c) frequency sweep (Tan (delta)). All at 10 °C.

Regarding the loss tangent or Tan(delta), when comparing the mean values at 10 °C (Fig. 4c), it was found the following decreasing order of values; TA (0.101 ± 0.015) > O (0.083 ± 0.007) > TO (0.059 ± 0.003) > TB (0.021 ± 0.003) ≈ B (0.019 ± 0.005) ≈ C (0.013 ± 0.005) with a statistically significant difference (P ≤ 0.05) among all samples, except between B and TB and C. This is an indication that bentonite and tannic acid decrease the stiffness

of the structure in the gelatin systems TA, O and TO, favoring a higher mobility, making them more "flexible" as result of their higher G''.

For all formulations, the observed Tan(delta) values at 10 °C and the shape of their profiles, are similar to those of amorphous polymers with high molecular weight, below their glass transition temperature, where the polymer chains are immobilized. Thus, the answer to an external stress implies local adjustments, something similar to the mechanical deformation of an ordinary solid (Ferry, 1980). This agrees with the theoretical model proposed by Joly-Duhamel *et al.* (2002), where the helices build the network itself and are not considered as the cross-links of the long flexible coils, in other words, it is like an entangled network of rigid rods (triple helix), which can be deformed through the flexible links (long flexible coils, dangling ends, loops attached to the network, or free chains), without bending of the rods.

The stress amplitude sweep at 25 °C (in the sol domain), shows that for all formulations, G'' > G' (Fig. 5a), and their values are stress independent over the applied stress range (0.1 - 1 Pa). No real breakage of the structure (no inflection point) can be detected. A linear viscoelastic domain can be clearly seen in the above mentioned stress range, thus, the value of 0.63 Pa was chosen to run the frequency sweeps.

In Fig. 5 b the profiles of the storage modulus (G') from the frequency sweeps run at 25 °C are seen. It is noticed on the one hand, that for the solution samples O, TB, B, TA and C, there is not a clear trend since the plots are frequency-dependent, overall G' increased with the frequency (Fig. 5b). On the other hand, for the formulation TO, its G' plot grew steadily with the frequency, and this modulus was much higher at low frequencies (0.1 a 6.1 Hz) than those of the other formulations, probably due to the combined effect of tannic acid and treated bentonite.

In Fig. 5c the profiles of the loss modulus (G'') from the frequency sweeps run at 25 °C are observed. It is seen that for all solutions, G'' showed a defined behavior with values higher than those of G'. When the plots were compared, it was seen that for G'' values; TO > O > TB > B > TA > C; observing that both moduli values (G' and G'') increased with tannic acid and treated bentonite addition, which suggests that overall, such components are enhancing the gelatin system structure, increasing especially the strength of the gels from the sample TO, because of a higher interaction among its parts, as compared
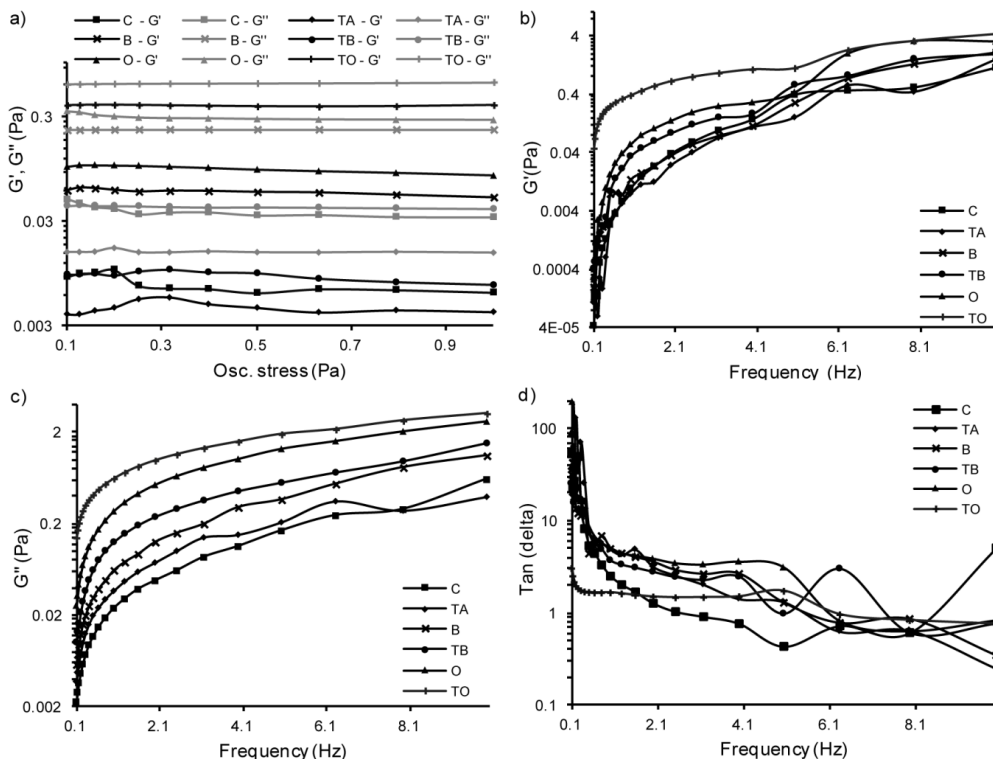
Fig. 5. Rheological profiles from gelatin solutions. C (100, 25, 0, 0,0), TA (100, 25, 8.47, 0, 0), B (100, 25, 0, 16.83, 0), TB (100, 25, 0, 0, 16.83), O (100, 25, 8.47, 16.83, 0) and TO (100, 25, 8.47, 0, 16.83) g / 4500 g water, prepared with gelatin, glycerol, tannic acid, control and treated bentonite respectively. a) Amplitude sweep (G', G''), b) frequency sweep (G'), c) frequency sweep (G'') and d) frequency sweep (Tan (delta)). All at 25 °C.
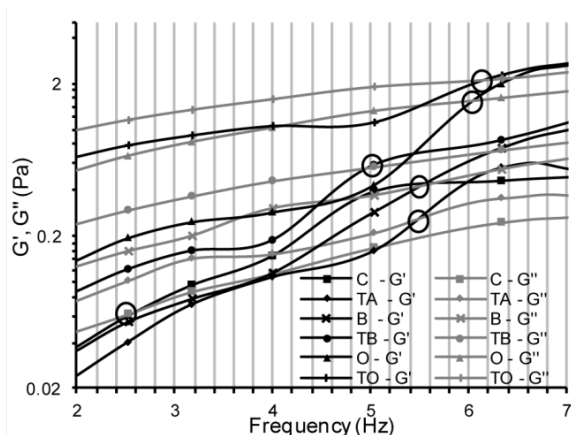

Fig. 6. Frequency sweep profiles from solutions C (100, 25, 0, 0,0), TA (100, 25, 8.47, 0, 0), B (100, 25, 0, 16.83, 0), TB (100, 25, 0, 0, 16.83), O (100, 25, 8.47, 16.83, 0) and TO (100, 25, 8.47, 0, 16.83) g / 4500 g water, prepared with gelatin, glycerol, tannic acid, control bentonite and treated bentonite respectively, showing the cross-over of G' and G'' at 25 °C.

when such constituents are added individually. Also, the particle size was fundamental to increase the interactions among all sample components, a decrease of 200 nm in the particle size between the control and the treated bentonite (Table 1), promoted a significant change in the viscoelastic properties of the gelatin systems.

For the gelatin solutions; the viscous modulus predominated (G'' > G') up to a cross-over (ω^*), i.e., a point where G' = G'' (Fig. 6), from this point onwards, the opposite trend was seen (G'> G''). In this cross-over, it is possible to obtain the relaxation time (T), described (Winter, 1987) as:

$$T = 1/\omega^* \qquad (5)$$

This parameter usually increases when the particle total volume increase in relation to the dispersion total volume (Tadros, 2011). On application of the above shown equation, the relaxation times found were $6.77\times10^{-2}\pm8.83\times10^{-3}$, $3.09\times10^{-2}\pm1.78\times10^{-3}$, $2.8 \times 10^{-2} \pm 1.15 \times 10^{-3}$, $2.70 \times 10^{-2} \pm 4.52 \times 10^{-3}$, $2.64\times10^{-2}\pm5.70\times10^{-4}$ and $2.52\times10^{-2}\pm5.70\times10^{-4}$ s

for the samples C, TA, B, TB, O and TO, respectively, with a statistically significant difference (P ≤0.05) between C and TA, B, TB, O and TO. The tannic acid and in a higher degree bentonite, decreased the relaxation time, probably by decreasing the gelatin molecular volume.

Following the classification proposed by Ferry (1980); for all formulations (Fig. 5d), according to the values and profiles of Tan(delta) at 25 °C; their behavior is similar to that of dilute polymeric solutions (Tan (delta) > 1), where it is considered that viscoelasticity is a relatively minor perturbation of the solvent Newtonian behavior, and the polymer molecules are sufficiently separated so as to move independently without much interaction.

3.2.3 Temperature sweep

The observed viscoelastic behavior of all solution samples (Fig. 7), is consistent with the results previously described for the six formulations. Below the transition temperature the storage modulus predominates (G' > G"); the moduli values (G', G") overall were higher than those shown by the solutions at higher temperatures, after such transition temperature the loss modulus predominates (G" > G').

The Tsol-gel found at the cross-over point (G' = G"), for the six formulations were TO (20.5 °C ± 0.3) > TB (19.8 °C ± 0.1) > B (18.8 °C ± 0.06) > O (17.5 °C ± 0.2) ≈ C (17.8 °C ± 0.06) > TA (15.1 °C ± 0.1), with statistically significant differences (P ≤0.05) among all samples, except between O and C. It is clearly seen that bentonite addition increased the Tsol-gel, with a higher effect of the treated material. It is also seen that the tannic acid decreased the Tsol-gel, about three degrees respect to the gelatin solution C.

Zhang *et al.* (2010a) found that at high concentrations the tannic acid works as a plasticizer, interacting by hydrogen bonds with gelatin, generating networks and interfering with triple helix links formation. This could explain the decrease in the transition temperatures, as well as the reduction at 10 and 25 °C of the gel strength (an increase in loss tangent at 10 °C, a decrease in G' at 25 °C). However, as observed in this work, when the tannic acid interacted with bentonite, different effects to those previously described were seen, the strength of gels increased (e.g., O and TO), suggesting that the tannic acid also exhibited interactions with the bentonite, giving place to gelatin- tannic acid -bentonite links.
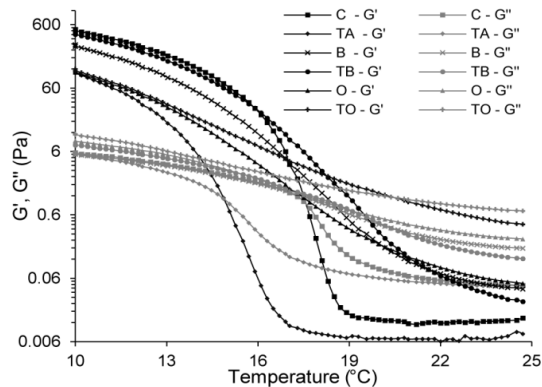

Fig. 7. Temperature sweep profiles (G', G") from solutions of C (100, 25, 0, 0,0), TA (100, 25, 8.47, 0, 0), B (100, 25, 0, 16.83, 0), TB (100, 25, 0, 0, 16.83), O (100, 25, 8.47, 16.83, 0) and TO (100, 25, 8.47, 0, 16.83) g / 4500 g water, prepared with gelatin, glycerol, tannic acid, control bentonite and treated bentonite respectively.

Conclusions

The composite materials generated by the mixture of bentonite, tannic acid, glycerol and gelatin; produced gels with higher strength and less temperature dependence, as compared to gels produced by each component individually. The particle size was crucial since a decrease of 200 nm, strongly affected the solution samples consistency and viscoelastic properties. Besides, it can be stated that there was an increase in the interactions between the various components, because when the particle size decreased, there was a higher bentonite surface area available to react with the other elements.

The tannic acid had a plasticizing like effect towards gelatin, reducing the storage modulus (G'), at 10 and 25 °C, however, when it interacted with bentonite, it increased both moduli (G', G") values, increasing the gels strength (samples O and TO), suggesting that it also presented interaction with bentonite, being generated links among gelatin-tannic acid-bentonite. Further research is needed to define all sorts of interactions among the various components studied, but this investigation has shown that the rheological properties of this sort of biocomposites, are a function of the temperature as well as the materials involved and its concentrations.

Nomenclature

C   control gelatin solution

TA   gelatin solution with tannic acid
B   gelatin solution with control bentonite
TB   gelatin solution with treated bentonite
O   gelatin solution with tannic acid and control bentonite
TO   gelatin solution with tannic acid and treated bentonite
AFM   atomic force microscopy
LVR   linear viscoelastic region
G'   storage or elastic modulus
G"   loss or viscous modulus
Tan(delta) loss tangent (G"/G')
Tsol-gel   gel point
Greek symbols
μ viscosity
τ shear stress (Pa)
γ shear rate (s^{-1})
k consistency coefficient (Pa.s^n)
n flow behavior index (dimensionless)
T relaxation time
ω^* cross-over point where G' = G"

Acknowledgments

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