Soft Matter

Volume 1 | Number 1 | Jan 2013 | Pages 1–100

Soft Matter

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Novel gigahertz frequency dielectric relaxations in chitosan films

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Abstract

Molecular relaxations of chitosan films have been investigated in the wide frequency range of 0.1-3×10^9 Hz from -10^0C to 110^0C using dielectric spectroscopy. For the first time, two high-frequency relaxation processes (in the range 10^8-$3 \cdot 10^9$ Hz) are reported in addition to the low frequency relaxations α and β. These two relaxation processes are related to the vibration OH and NH_2/NH_3^+, respectively. The high-frequency relaxations exhibit Arrhenius-type dependencies in the temperature range 10°C to 54°C with negative activation energy; this observation is traceable to hydrogen bonding reorientation. At temperatures above the glass transition temperature (54^0C), the activation energy changes from negative to positive values due to breaking of hydrogen bonding and water loss. Upon cooling in a sealed environment, the activation energies of two relaxation processes are nearly zero. FTIR and XRD analyses reveal associated structural changes upon heating and cooling. These two new high-frequency relaxation processes can be attributed to the interaction of bound water with OH and NH_2/NH_3^+, respectively. A plausible scenario for this high-frequency relaxations is discussed in light of impedance spectroscopy, TGA, FTIR and XRD measurements.

Key words: *Biopolymer, Chitosan; dielectric spectroscopy; high frequency dielectric relaxation; negative activation energy*.

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Introduction

Chitosan is a polysaccharide which is a N-deacetylated derivative of chitin. After cellulose, chitin is the most abundant natural polymer. Chitosan has several favorable properties, such as biodegradability, biocompatibility, and non-toxicity; it exhibits better solubility in acidic aqueous media than chitin.[1] Chitosan is easily processed into films, gels, nanofibers, nanoparticles and scaffolds, thus it is used in advanced biomedical and pharmaceutical applications, tissue engineering, controlled drug delivery, biotechnology, and in food industry.[2-4] For some applications such as antibacterial material, non-neutralized film (acetate form) is preferred due to the presence of acetic acid that significantly increases antibacterial response.[5] Moreover, by having dilute acetic acid in the chitosan (non-neutralized film), it exhibits a remarkable ability to reduce metal ions ($AgNO_3$, $HAuCl_4$) into metal nanoparticles with low particle size distribution.[6] Additionally, the ionic conductivity of chitosan-acetate, it can find applications in alkaline polymer electrolytes fuel cells.[7,8] In other applications such as tissue regeneration or cell adhesion and proliferation, chitosan films must be used in the neutralized form.[9]

Because of all the above applications, it is important to fully characterize the properties of chitosan (neutralized and non-neutralized) in solid state, its physical, physicochemical and dielectric relaxation, including the molecular mobility that occurs within the polymeric structure in both wide frequency and temperature ranges.

Dielectric spectroscopy is one of most commonly used techniques for investigation of glass transition phenomena which is related to α-relaxation.[10] For polymers, α-relaxation corresponds to the segmental motion of chains.[11] Additionally, inter- and intra-molecular interactions can give rise to the appearance a new relaxation process in polymer materials which can be detected using dielectric spectroscopy.

Refs. [12-16] reported the dielectric relaxations found in variety of polysaccharides that exhibit similar relaxation processes. The β-relaxation is the main one found in all polysaccharides, including chitosan, at temperatures between -135 to 120° C; this relaxation is related to segmental motions of the chains via the glycoside bond and therefore, it corresponds to local chain dynamics appearing just above 10^6 Hz.[17] The σ-relaxation process at higher temperature (80-180[0]C) and low frequency range (about of 100-1000 Hz) is associated with the hopping motion of ions in the disordered structure of the biomaterial [17,18] and it is independent on water content. Additionally, an α-relaxation assigned to the motion of a water-polymer complex has been

observed in wet chitosan and wet cellulose materials in the Hz range between 20-70^{0}C; this relaxation is traceable to a plasticizing effect of water.[18-20] It is noteworthy that in hydrated samples of polysaccharides, proteins and biopolymers water molecules can be directly bounded to give rise to additional relaxations.[21,22] The polymer chain restricts the orientation of water molecules such that the collective motions of the macromolecule-water are distorted giving rise to relaxation times about 100 times as slow as that of pure water.[22]

It is noteworthy that the physical and chemical properties of chitosan can be significantly changed by the presence of small amounts of water.[23] The increasing numbers of studies of dielectric measurements in polysaccharides are controversially discussed about the shape of the dielectric spectra, number of relaxation process and their interpretation.[17,18,23,24] Among the main causes for the discussion are the very complex supramolecular structures of polysaccharides such as intra and intermolecular hydrogen bonds and the strong influence of water content [25] that can be responsible for additional relaxation process in the material in the high frequency range. It has been previously reported a dielectric relaxation at 1 GHz frequency for different hygroscopic solids.[26] This relaxation can be attributed to the breakage of hydrogen bonds between adjacent water molecules in thin films in microwave frequencies. The high frequency dielectric measurements of aqueous solutions and dynamical structure of free water around biopolymers also have been reported.[22] However, to our knowledge, there is no report in the literature about the molecular origin of the dielectric relaxation properties of biopolymer films in the gigahertz (GHz) frequency range. High frequency dielectric relaxations are important to fully understand the physicochemical and electrical properties of polymer material including the molecular mobility, dielectric relaxation and hydrogen bonding structure. Dielectric spectroscopy is a relevant technique to probe translational or rotational molecular motions and hydration properties with high sensitivity.

Additionally, chitosan is semicrystalline material; the crystal structure of the chitosan polymorph was first reported by Sakurai et al.[27] Later Ogawa et al. reported that tendon morph was commonly observed with hydrated films in both non-neutralized and neutralized films.[28] Upon heating the hydrated chitosan or tendon forms were converted into anhydrous or annealed form.[29] Such changes in chitosan structure in the heating process can be affected on relaxation properties of material.

The objective of this work is to investigate the temperature-dependent dielectric relaxation behaviors of non-neutralized and neutralized chitosan films in the frequency range of 1 MHz to 3 GHz and temperature range of -10 to 110°C using dielectric spectroscopy. Additionally, interpretation of dielectric spectra can be greatly aided with the use of complementary techniques such as TGA, FTIR and XRD measurements. All these techniques may prove useful in determining structural changes that undergo polymer films upon heating/cooling cycles.

Materials and Methods

Materials

Chitosan (CS), 86% of degree of deacetylation (DD) and molecular weight of *ca.* 350 kDa was purchased from Sigma- Aldrich and was used as received. CS films were obtained by dissolving 1 wt % of CS in a 1 wt % aqueous acetic acid solution with subsequent stirring to promote dissolution. Films were prepared by the solvent cast method by pouring the solution into a plastic Petri dish and allowing the solvent to evaporate at 60°C. To obtain the neutralized films and to remove acetic acid, chitosan films were immersed into a 0.1M NaOH solution during 30 min and washed with distilled water until neutral pH; a subsequent drying step in furnace at 60°C for 14 hours was performed. A thin layer of gold was vacuum-deposited onto both film sides to serve as electrodes.[18]

Methods

Dielectric spectroscopy measurements were carried out using three Impedance Analyzers: Solartron 1260 (in the frequency range 1-10^4 Hz); Agilent 4249A (in the frequency range 10^2-10^7 Hz) and Agilent E4991A (in the frequency range 10^6-3x10^9 Hz). The frequency measurements were carried out in the cell in the bench-top temperature chamber SU-261 with controlling temperature from -10^{0}C to 110^{0}C. This configuration has the advantage of providing high precision temperature control and impedance measurements over the entire frequency range. Measurement at the each temperature was done after 5 min exposed to ensure thermal equilibrium. Supporting evidence was obtained from FTIR spectroscopy, (Perkin–Elmer) using an ATR accessory in the range 4000– 400 cm^{-1}; resolution was set to 4 cm^{-1}; it is equipped with

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MiRacle Heated ZnSe system and *in situ* temperature controller in the temperature range of 25°C to 110°C.

Water content was evaluated by using a TGA Mettler Toledo apparatus, model TGA/SDTA 851e under dry air atmosphere. Heating rate was set to 10°C/min. It is well known that chitosan is a mixture of amorphous and crystalline phases.[30, 31] Such that additional *in situ* X-ray diffraction measurements were carried out using a Rigaku Ultima-IV diffractometer with temperature chamber (Rigaku HT-1500) to evaluate structural changes as a function of temperature.

Results

Dielectric measurements

Typical dependence of dielectric loss ε" with frequency (1Hz to 3 GHz) of as-prepared non-neutralized and neutralized chitosan films (with water content about 11 wt % obtained from TGA measurements) are shown on Figure 1 at the two selected temperatures indicated on graphs. These dependencies have been obtained on three Impedance Analyzers. These spectra have been calculated from dielectric spectroscopy measurements using DC correction as described elsewhere.[12, 18] Figure 1 shows three relaxation processes: 1) low frequency α-relaxation, 2) β-relaxation (10^5-10^8 Hz) and 3) two additional new high frequency relaxation processes (10^8-$3 \cdot 10^9$ Hz).

It is noteworthy that α-relaxation observed in non-neutralized films has higher amplitude and it is observed at higher frequency range when compared with neutralized films. This effect can be related to fact that the α-relaxation process is highly dependent on acid content which changes viscosity of chitosan solution and act as plasticizer.[32, 33]

Figure 2 shows a typical dependence of the dielectric loss (ε″) with frequency of the as-prepared non-neutralized chitosan films at four different temperatures. The fitting of the complex permittivity in non-neutralized and neutralized films were carried out using the well-known Cole-Cole empirical model.[34]

$$\varepsilon^* - \varepsilon_\infty = \frac{\varepsilon_s - \varepsilon_\infty}{[1 + (jw\tau)^{1-\alpha}]}$$

where ε_s-ε_∞ and ε_∞ are the dielectric relaxation strength and the dielectric constant at the high frequency limit, respectively; τ is relaxation time. The exponents α introduce a symmetric broadening of the relaxation.

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Due to non symmetrical shape of the dependencies of the dielectric loss with frequency it is customarily to dissect the impedance response as the sum of two Cole-Cole contributions. As one can see from Fig. 2, the sum of two Cole-Cole peaks fits well the overall behavior (continuous lines; this gives rise to unsymmetrical shape). For illustrative purposes we show the deconvolution of the data at 4°C as the sum of two Cole-Cole peaks (tagged as p1 and p2).

The dielectric loss (ε'') shows two maxima at frequencies *ca.* 5.8 10^8 and 1.3 10^9 Hz for non-neutralized chitosan polymer films; a similar behavior have been observed and in neutralized films.

Figures 3a and 3b show that the dependencies of real part of permittivity measured at the frequency 1 MHz versus temperature for as-prepared wet and dry non-neutralized and neutralized chitosan films in the process of heating and cooling. This frequency has been chosen to avoid Maxwell-Wagner interface and electrode polarizations which is observed at high temperature in chitosan films below 10^5 Hz.

It is noteworthy that after heating to 110^0C, films were annealed at this temperature for 30 min to drive off any moisture. This procedure ensures that measurements during cooling were carried out on dry samples with water content less than 0.5 wt % (according to TGA measurements, insert on Fig. 3). As one can see real part of permittivity (or dielectric constant) for both wet films exhibit a peak at *ca.* 54^0C. Conversely, during the cooling process (dry films) dielectric constant decrease with decreasing of temperature.

Figures 4 and 5 show the dependencies of relaxation time *versus* reciprocal temperature for as-prepared wet non-neutralized and neutralized chitosan films in the process of heating and cooling.

Below 54^0C in both non-neutralize and neutralize films, the temperature dependence of relaxation time τ_1 of process 1 can be described by Arrhenius-type dependence: $\tau = \tau_0 \exp\frac{E_a}{RT}$, with negative activation energy E_a. The values of E_a are -3.02±0.15 and -3.54±0.3 kJ/mol in non-neutralize and neutralize films, respectively. For higher temperatures, relaxation times decrease and E_a values change from the negative to positive with activation energy +3.41±0.17 kJ/mol and +3.89±0.35 kJ/mol for non-neutralize and neutralize films, respectively.

For process 2 below 54^0C in non-neutralize films, the temperature dependence of relaxation time τ_2 is close to zero and at higher temperatures it becomes positive with activation energy

+1.72±0.15 kJ/mol. In contrast, in neutralize films the relaxation time of process 2 at the temperature 54⁰C changes value from negative (with Ea=-3.35±0.3 kJ/mol) to positive (with Ea=+4.61±0.2 kJ/mol).

During the process of cooling on both dry-non-neutralized and dry-neutralized films, the temperature dependence of relaxation times of process 1 and 2 exhibit practically zero activation energies.

DC conductivity

The values of DC (direct current) conductivity have been obtained from fitting of impedance spectra in low frequency range 1-10^4 Hz using Solartron 1260 and methodology descried in ref.[9] Fig. 6 shows dependence of DC conductivity σ versus 1000/T for wet (as prepare), and after annealed at temperature 110⁰C for 30 min in the process of cooling for non-neutralized (Fig. 6a) and neutralized (Fig. 6b) films.

Both non-neutralized and neutralized films show similar behavior: nonlinear dependence in wet samples and two linear dependencies in dry films in the cooling processes. Nonlinear dependence is a typical feature of the α-relaxation behavior related to glass transition.[11, 18] The temperature dependence of conductivity is well described by the Vogel-Fulcher-Tammann-Hesse (VFT) equation.[35]

$$\sigma = \sigma_0 \exp(-\frac{DT_0}{T-T_0}),$$

where D is a constant and T_0 denotes the Vogel temperature.

Results of fitting using VFT equation are shown on Fig. 5 as continuous lines. From this fitting the Vogel temperature is calculated as 277.1±1.2 K and 278±1.3K for non-neutralized and neutralized films, respectively. The same value of Vogel temperature has been obtained from dielectric spectroscopy measurements by fitting dependence of relation time of α process versus reciprocal temperature. [18]

T_0 in many amorphous and semicrystalline polymers is usually 50⁰C lower than glass transition temperature, Tg.[11, 18] Using this empiric relation Tg can be calculated as 54.1±1.2⁰C and 55±1.3⁰C for non-neutralized and neutralized films, respectively.

In dry films during the cooling process the α-relaxation has not been observed because this relaxation is related to a plasticizing effect of water.[18-20] In dry samples and upon cooling from 105 to 46⁰C, the DC dependence of the conductivity is well described by the Arrhenius model for

both neutralized and non-neutralized chitosan. This relaxation, so-called σ-relaxation, has been widely studied and it is associated with the hopping motion of ions in the disordered structure of the biomaterial.[17, 18] Further cooling from 46 to 20^0C, the DC dependence of conductivity with temperature in chitosan can be represented by the variable range hopping model proposed by Mott.[36]

Infrared Spectroscopy

To understand mechanisms of interaction of the bound water molecules with chitosan film as a function of temperature additional FTIR measurements were carried out on non-neutralized and neutralized films under the same heating-cooling cycles (analogously to dielectric spectroscopy and TGA measurements). Figures 7a and 7b show FTIR spectra of the non-neutralized chitosan film in the process of heating and cooling. Firstly, the non-neutralized chitosan film spectra (see Fig. 7a) is observed by confirming the appearance of the $-NH_3^+$ band at 1545 cm^{-1} (note that there is no apparent $-NH_2$ band at 1590 cm^{-1} in neutralized films). One may conclude that this film is essentially in the acetate (non-neutralized) form.[37] The characteristic 3700-3000 cm^{-1} vibration bands corresponds to the amine and hydroxyl groups and the characteristic peaks at 1639 cm^{-1} (amide I group) and 1549 cm^{-1} (bending vibrations of NH_3) are also observed.[38] The absorption band centered at 3350 cm^{-1} (Fig. 7a) confirms the presence of broad O-H and N-H stretching in which the O-H bands overlap by N-H stretching.[39]

In case of non-neutralized films, during the process of heating, there are two relevant changes observed: 1) reduction of intensity and broadening of the band centered at 3350 cm^{-1} which shifts to 3445 cm^{-1} (upon heating); and 2) the band at 1635 cm^{-1} shifts to 1654 cm^{-1}. These changes could be affecting the hydrogen bonded structure of the chitosan film due to elimination of adsorbed water [25] and the breaking of intra-chain hydrogen bonds between water molecules and NH or OH groups of the chitosan molecule. It is noteworthy the appearance of a new band at 1710 cm^{-1} above 70^0C. This new band at 1710 cm^{-1} is ascribed to the interaction of NH_3^+ groups of chitosan with $-COO^-$ in the process of drying of film.[39]

Fig. 7b shows FTIR spectra of non-neutralized chitosan films during the process of cooling. The absorption band at 3445 cm^{-1} has a relatively constant intensity and broadening. This observation indicates a possible overlapping of hydrogen bonding with –OH and NH groups Additionally, the band at 1654 cm^{-1} shifts to 1649 cm^{-1}.

Figures 8a and 8b show FTIR spectra of neutralized chitosan film during the heating and cooling cycles. The most important feature of the FTIR spectra of neutralized films is the presence of a band at 1590 cm^{-1} which corresponds to the N-H stretching of the amine group. The removal of acetate counter ion (by neutralization) is further confirmed by the appearance of the bands at 1419 cm^{-1}, 1375 cm^{-1} and 1320 cm^{-1} corresponding to CH$_2$ bending, CH$_3$ deformation and CH bending, and CH$_2$ wagging, respectively.[40] For neutralized films upon heating (Fig. 7a), the broad band centered at 3360 cm^{-1} shifts to 3450 cm^{-1} with significant reduction in intensity and the band at 1655 cm^{-1} shifts to higher wavenumber values. The shift of these characteristic vibrations to higher wavenumbers and decrease in intensity is associated with elimination water from neutralized film.[25] It is noteworthy that in neutralized film the peak at 1710 cm^{-1} has not been observed.

In the cooling process (Fig. 8b), the band centered at 3450 cm^{-1} shifts to 3360 cm^{-1} with increasing of intensity and the band at wave number 1655 cm^{-1} shifts to lower wave number. These changes are likely due to new types of hydrogen bonding in the swollen chitosan environment. It is notable that for neutralized films there is a large increase of intensity of the band at 3360 cm^{-1} during the cooling process.

The changes observed in the FTIR spectra for both non-neutralized and neutralized films in the heating-cooling process will help explain the temperature-dependence of relaxation times obtained in impedance measurements.

X-Ray Diffraction

The X-ray diffraction patterns of chitosan non-neutralized film under different temperatures are presented on Fig. 9. At room temperature (25 °C), the corresponding diffractogram shows a mixture of two crystalline phases and an amorphous one. After applying a whole-pattern deconvolution with Gaussian peaks, the area below the broadest peak (with a center located at $2\theta = 18° \pm 0.05°$) is associated to amorphous fraction (*ca.* 67%). On the other hand, crystalline fraction is characterized by two hydrated crystalline phases: *tendon* or hydrated Form I (hereafter *T*), and hydrated *L*-2 or Form II (hereafter *L*-2) which is observed in non-neutralized acetate films.[28] The procedure for indexing them is based on the calculated diffraction pattern extracted from the structures modeled in reference 30. Phase *T* is usually reported as the stable polymorph in chitosan systems, either if they are neutralized or not.[31] In the present work the distinguishing

reflections of T are: (001), (110), (200) and (032). Three peaks of phase L-2 have been identified in pattern at room temperature as: (003), (200) and (121). It is a metastable phase and previous studies[30] noted that it spontaneously transforms into the phase Annealed (hereafter An).

On heating, the occurrence of the An phase is detected from 60 °C up to 105 °C. This transition (i.e., T —> An) is concomitantly noticed with an increasing of intensity and sharpening of the complex peak at central part of XRD patterns. It is presumed that this behavior is not only a direct consequence of the change in the crystal structure and decreasing the amount of amorphous fraction (about 57% at 105 °C), but it is chiefly caused by the progressive increasing of the An fraction (peaks (110) and overlapped reflections (200) with respect to those of the hydrated phases L-2 (reflections (200). Also there is an intensity decrease in direction (003) and T phase (decrease reflections (110) and the disappearing of peak (032). Table 1 shows the association of the (hkl) indices with the corresponding 2θ positions and the different phases encountered (i.e., T, L-2 and An).

The decreasing of the amount of amorphous phase (or the crystallization process) on heating is caused by the loss of water as reported in Reference 41. It is important to emphasize that the predominant phase at high temperature is An and that it is a product of three dynamical related-processes: 1) non-isothermal crystallization of amorphous fraction by progressive loss of weakly-bonded water on heating; 2) transition from T phase to An phase, and 3) spontaneous transition from metastable L-2 phase to An phase. In reference to this latter transition, it is restrained during the heating process in view of the persistence of the characteristic reflection (003) of L-2 up to 105 °C (Fig. 9). In a first approximation, a likely explanation for this phenomenon is the competition between water and acetic acid during hydrolysis of non-neutralized chitosan acetate films. In other words, an increment of the loss of water molecules obstructs the loss of acetic acid and, subsequently, the permanence of that limits the advance of transition $L2$ —> An. In this sense, the kinetic of this latter transition is directly related with the dependence of non-neutralized chitosan hydrolysis on water content in the films.[42]

Lack of L-2 Form in neutralized films (Fig. 9b) directly relates with the absence of acetic acid[42] that increases amorphous fraction (about 70% at 25 °C and 63% at 105 °C). Thus, the crystallization of neutralized films by heating is only represented by two processes 1) non-isothermal crystallization of amorphous fraction by progressive loss of weakly-bonded water on heating and 2) transition from T phase to An phase.

It is noteworthy that after annealing at the temperature 110^0C during 30 min in the diffractograms of cooled samples at 25^0C (Fig. 9, upper dependencies), there are not significant changes when compared with diffractograms obtained at 105^0C. This means that in the cooling process, high water absorbance does not take place; this observation is confirmed by TGA measurements (water content in the annealing samples is about 0.5%).

We note that all of our previous measurements (DC, X-ray and TGA) were run in the same heating-cooling cycles (analogously to dielectric spectroscopy). This strategy will help understand molecular structure changes upon heating-cooling and explain features of the high frequency relaxation.

Discussion

High frequency dielectric measurements in chitosan films allowed to observed two new relaxation processes in the frequency range 10^8-$3 \ 10^9$ Hz which exhibit a complex dependence on temperature. Upon heating below 54^0C a negative activation energy has been observed. However, for temperatures higher than 54^0C activation energy changes from the negative to positive (Figs. 4 and 5). Negative activation energy at the frequency of *ca.* 300 MHz has been observed in hydrated lysozyme[43, 44] and serum albumin[44]; in hydrate collagen at *ca.* 200 MHz [45] and *ca.* 100 kHz [46]; β-cyclodextrin complex with 4-*t*-butylbenzyl alcohol at *ca.* 1 MHz [47] and calcified and decalcified bone at *ca.* 100 kHz [48]. Despite many studies of different materials, the explanation of observed negative activation energy has been based on the suggestion that the activation process involves the breaking of a hydrogen bond between surrounding molecules and water molecules in their primary hydration shell. This hydration shell is followed by the formation of other hydrogen bonds between the reorienting water molecule and its neighbors[49] or the formation of more stable hydrogen bonds between reoriented water molecules.[47] However, explanations proposed in the literature could not be directly applied to chitosan films due to a more complex dependences of activation energy on temperature (changing the slope from the negative to positive values) and the high frequency range (between 10^8-$3 \ 10^9$ GHz). It is noteworthy that the relaxation frequency of bound water in different biomaterials is typically found at 100 MHz, whereas the relaxation of free water is *ca.* 16 GHz.[50]

It is well known that water molecules can be bonded through hydrogen bonding with both -OH and -NH$_2$ (or NH$_3^+$ in non-neutralized) groups of chitosan.[18,25] According to FTIR

measurements for the entire temperature range, the most significant changes are observed in absorption band related to these groups. One can conclude that these groups are responsible for observed high frequency relaxation processes in both non-neutralized and neutralized films.

An important question arises: why does the activation energy changes from negative to positive at temperatures above 54°C? According to DC conductivity measurements this value equals to the glass transition temperature (54.1 ± 1.2^{0}C and 55 ± 1.3^{0}C for non-neutralized and neutralized films, respectively).

Additionally, the confirmation that this temperature corresponds to the glass transition is the appearance of peaks about 50°C which has been observed in wet films by analyzing the dependence of ε' versus temperature (Fig. 3). To make sure that this peak has no relation with water evaporation, a simple estimation has been performed using the Maxwell-Wagner effective medium model that considers a single inclusion (in our case water with ε_2) surrounded by continuous matrix of polymer (with ε_1). This model is often used in the polymer composites for estimation of effective dielectric constant $\varepsilon_{eff.}$[51] Due to the relatively low water content about 10 wt. %, this approximation can give a realistic estimation. The Maxwell-Wagner equation is given by.[51]

$$\varepsilon_{eff} = \varepsilon_1 \frac{\varepsilon_2 + 2\varepsilon_1 - 2(1-\phi_1)(\varepsilon_1 - \varepsilon_2)}{\varepsilon_2 + 2\varepsilon_1 + (1-\phi_1)(\varepsilon_1 - \varepsilon_2)}$$

were $1-\Phi_1$ is volume fraction of water in the chitosan matrix.

To put our results in perspective, we now calculate the temperature-dependence of effective dielectric constant at the frequency 1 MHz of chitosan films according to Maxwell-Wagner equation. In this calculation we use temperature dependence of water dielectric constant (ε_2) [52] and by taking into account that dielectric constant of water at the frequencies below 1 GHz practically constant [53, 54] and it only depends on temperature.[54] To calculate the temperature-dependence of ε_{eff} at 1 MHz, we take ε_1 value as that of dry chitosan films (see Fig. 3, open circles). Water volume fraction was obtained from TGA measurements. This calculation gives rise a monotonically dependence on the real part of permittivity with decreasing of water content (continuous lines on Fig. 3). The same estimation has been performed for calculating the effective conductivity of chitosan-water complex by taking into account that the conductivity of ultra pure water is *ca.* 5.5 10^{-8} S/cm. The results obtained have shown that the water evaporation practically do not affected on conductivity of chitosan films.

Consequently the peak observed in the dependencies of ε′ versus temperature can be related to the glass transition. It is well known that when the temperature increases above the glass transition temperature, ε′ typically exhibits peaks that tend to decrease with a further temperature increasing. This type of behavior is due to the random thermal motions of chains which makes it more difficult for dipoles to align in the direction of an applied electric field.[50, 55] Conversely, in dry films and upon cooling, those peaks and the glass transition have not been observed due to water evaporation (water act as plasticizer in the wet material).[18-20]

For amorphous materials, the glass transition temperature (T_g) is the temperature that separates glassy and rubbery states. As result, at T_g pronounced changes are observed in the mechanical, thermal and dielectric properties. In the hydrophilic chitosan, at the temperatures higher than T_g, there is an additional dehydration process (TGA measurements, insert on Fig. 3) and transformation of crystalline phase due to water evaporation (transition from hydrated to annealed form and increasing volume fraction of crystalline phase, Fig. 9). All these effects responsible for changes in the thermal motions of NH and OH chains.

It is noteworthy that the well-known model of thermal dipolar relaxation is based upon the presence of two states of dipolar group orientation separated by potential-energy barrier which approximately equals activation energy.[56] Molecular dynamics of site groups in chitosan is not well known; however, the molecular dynamics simulation of hydroxymethyl side groups in cellulose [57] and OH side groups in poly(vinyl alcohol) (PVA)[58] considers a motion of these groups between two sites separated by an energy barrier which appears due to the presence of intermolecular and intramolecular hydrogen bonds. For example, in PVA the presence of intermolecular and intermolecular hydrogen bonds are responsible for hydroxyl side groups to have two energy minima with difference in energy of 5.5 kJ/mol and only 8% of groups have average energy 10 kJ/mol.[58] Consequently, this model suits well for explaining experimental results in chitosan films.

At temperatures below glass transition temperature (54^0C), the initial structure of wet films, according to XRD measurements, are likely tendon structure consisting of a polymeric water structure comprised of a network of water molecules that are hydrogen bonded.[18, 25]

To identify the nature of the two observed relaxation processes it is important to emphasize on the chemical structure of chitosan; it has an -OH and a -NH₂ (neutralized form) and NH₃⁺ (non-neutralize films). The relaxation process 1 in both films centered at the frequency *ca.* 5.8

10^8 Hz and according to Fig. 4a and 4b has approximately the same negative and positive activation energies. Therefore, it is possible to conclude that process 1 relate to relaxation of -OH side groups (note that these groups are relatively unchanged by the neutralization of films). These conclusion is also confirmed by FTIR data: a similar shift of characteristic band associated with the –OH stretching vibration from 3350 cm^{-1} to 3445 cm^{-1} has been observed for both non-neutralized and neutralized films. It is noteworthy that PVA also possess OH side groups and the relaxation frequency of such groups is centered at *ca.* 10^8 Hz.[59]

In contrast, the relaxation process 2 is observed at the frequency *ca.* 1.3 10^9 Hz for both non-neutralized and neutralized films; however, they exhibit different dependencies on the temperature and activation energies. These differences can be related to the mobility of different side groups that change during the neutralization of films: NH$_2$ (neutralized) and NH$_3^+$ (non-neutralized). Additionally, it must be considered the presence of acetic acid in the non-neutralized film and the interaction of the COO$^-$ groups with the NH$_3^+$ groups of chitosan.[60] This interaction can be detected by FTIR measurements: appearance of new band at 1710 cm^{-1}. Therefore, one may conclude that relaxation process 2 can be related to relaxation of NH$_2$/NH$_3^+$ side groups.

We now discuss the nature of observed negative activation energy. The negative activation energy can be related to the reorientation of weak intermolecular hydrogen bonds between bound water and NH$_2$/NH$_3^+$ and OH groups; this changes the build-up symmetry of hydrogen bonding of the side groups NH and OH and as a result, energy of these groups is lowered.[61] We now give a plausible scenario to help explain the observed phenomena.

1. Fig 10 shows the schematic of proposed structure of neutralized (Fig. 10a) and non-neutralized (Fig. 10b) chitosan films below 54^{0}C. According to the literature [29-31, 38], hydrated form of chitosan has bonded and free water in chitosan matrix. Additionally, non-neutralize films have COO$^-$ groups which interact with the NH$_3^+$ groups of chitosan.[58] Upon heating (below glass transition), water molecules rearrange in such a way that build-up of hydrogen bonding is affected, i.e. the symmetry of hydrogen bonding changes. This event is thermodynamically favorable such that it frees side group mobility to evolve to another state. Consequently, a negative (favorable) activation energy is observed. Such effects have been observed in proteins: hydrogen bonds of hydroxyl groups with side groups of protein can generate molecular asymmetry in hydrogen bonding build-up with difference in energy of *ca.* 2.5 kJ/hydrogen bond.[59] This energy is higher than the potential energy in equilibrium state such that lateral NH$_2$

and OH groups pushes the system to a lower energy state; this relaxation is responsible for observed negative activation energy. We propose that only reorientation of hydrogen bonds with side groups of chitosan are promoted upon heating (not broken hydrogen bounds) as probed by FTIR spectra; it is observed that only: 1) a small reduction of intensity of band centered at 3350 cm^{-1} and band at 1655 cm^{-1} and 2) a small shift of T peak (110) of hydrated Form I to the higher 2θ observed in XRD spectra.

2. In the temperature range 54^{0}C (glass transition) to 110^{0}C, activation energy of relaxation process changes slope from negative to positive values. At a temperature above the glass transition the amplitude of the random thermal motions of chains increase leading to breaking of weak hydrogen bonds between water molecules and NH or OH groups of chitosan (Fig. 10c and 10d). Additionally, according to TGA measurements (Fig. 3, insert) the loss of water molecules has been observed. XRD measurements have shown the change in the crystal structure (from hydrate to annealing forms) by decreasing the amount of amorphous fraction and decreasing of interchain distances due to loss of water. The breaking of the H-bonds between water molecules and side groups of chitosan were responsible for: the high reduction of intensity and shift to higher values of band centered at 3350 cm^{-1}; reduction and shift to higher values of the band at 1635 cm^{-1} for non-neutralize films (and band at 1655 cm^{-1} in neutralized films). We propose that at temperatures above 54^{0}C (glass transition), side groups of chitosan posses energies higher than potential energy barrier between the two states: hydrate to annealing forms. Theses changes are due to the breaking of hydrogen bonds, water loss and as a result, the relaxations of the lateral groups exhibiting normal process with positive activation energy.

3. In the process of cooling, the activation energies of both relaxation processes are practically about zero or exhibit a very little negative slope (less than -0.02 kJ/mol). According to TGA data (insert on Fig. 3), it has not been observed a significant water absorbance (water content in the annealing samples is about 0.5%). According to XRD measurements (Fig. 9) it has not been observed changes in the crystalline structure of chitosan films (it practically does not change XRD pattern when compared with patterns at 105^{0}C). Dielectric constant in the cooling process decrease with decreasing of temperature (Fig. 3) due to lower freedom of movement of dipole molecular chains. FTIR spectra for both neutralized and non-neutralized films in dry and wet state at 30^{0}C showed a difference due to the different types of hydrogen bonding in dry and

wet films. The measurements in the cooling process confirmed the determining role of H-bonding and free water in the relaxation processes in chitosan films.

Based upon the above analyses, it is possible to conclude that two high frequency relaxation processes are related to the changes of energy and freedom of movement of the side groups OH and NH_2/NH_3^+, respectively.

Conclusions

The temperature dependence of high frequency (in GHz range) dielectric relaxation behavior of non-neutralized and neutralized chitosan films have been investigated using dielectric spectroscopy, TGA, FTIR and XRD measurements. For the first time, two new relaxation processes in the gigahertz frequency range has been reported.

The relaxation process centered at the frequency *ca.* $5.8 \ 10^8$ Hz relate to the mobility of OH side groups. The relaxation process observed at the frequency *ca.* $1.3 \ 10^9$ Hz related to mobility of NH_2/NH_3^+ side groups.

In wet films, glass transition phenomena is observed by performing temperature-dependent measurements of DC conductivity and dielectric constant. Upon heating and below the glass transition temperature, chitosan films exhibit an Arrhenius-like dependence with negative activation energy due to the disruption of the symmetry of hydrogen bonding (not H-bonding breakage). Upon further heating (above the glass transition) the activation energy changes slope from negative to positive because of water molecules are expelled from the vicinity of chain molecules and H-bonding breakage. After this thermal treatment, films are cooled to *ca.* 10°C and the activation energy is practically zero. FTIR spectra for both non-neutralized and neutralized films in the heating-cooling process have shown changes in vibration frequencies and intensities of side OH, and NH_2/NH_3^+ groups, respectively. XRD analysis reveals associated structural changes upon heating and cooling in the following fashion: 1) non-isothermal crystallization of amorphous fraction by progressive loss of weakly-bonded water on heating (above glass transition), and 2) transition from *Tendon* phase to *Annealed* phase. Based upon these changes in the chitosan structure the new model of high frequency relaxation has been proposed which can be attributed to the interaction of the bounded water molecules and the changes of energy and freedom of movement of side groups, namely OH and NH_2/ NH_3^+. It is noteworthy that in many applications, OH and NH_2/NH_3^+ groups strongly interact with water molecules but also in the formation of chitosan-based composites and nanocomposites. In many

cases, the nature of the molecular interaction of side groups with other polymers and nanoparticles is still not known. Therefore, the proposed high frequency method and model for the determination of energy and freedom of movement of side groups will allow to understand mechanism of interaction of chitosan with polymers and nanoparticles.

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Figure Captions

Figure 1: Dielectric loss factor (ε'') *versus* frequency for (a) non-neutralized and (b) neutralized chitosan films at the two indicated temperatures.

Figure 2: Dielectric loss factor (ε'') *versus* frequency for non-neutralized chitosan film measured at the indicated temperature (dashed-lines: fitting using Cole-Cole model).

Figure 3: Dependence of ε' on the temperature measured at the frequency 1 MHz on as-prepared (wet) and dry films (a) non-neutralized and (b) neutralized chitosan films. Continuous lines are results of fitting using Maxwell-Wagner equation. Insert shows TGA measurements on as-prepared film during first and second scans. Second scan have been obtained after annealing at the temperature 110^0C during 30 min.

Figure 4: Temperature dependence of relaxation time *versus 1/T* for non-neutralized film a) for first relaxation process (p1), and b) for second relaxation process (p2). Lines represent Arrhenius-type dependence fitting.

Figure 5: Temperature dependence of relaxation time *versus 1/T* for neutralized film a) for first relaxation process (p1) and b) for second relaxation process (p2). Lines represent Arrhenius-type dependence fitting.

Figure 6: Temperature dependence of DC conductivity *versus 1/T* for (a) non-neutralized film (b) neutralized film. Lines represent fitting using VFT equation.

Figure 7: FTIR Spectra of non-neutralized chitosan film (a) in the process of heating and (b) in the process of cooling.

Figure 8: FTIR spectra of neutralized chitosan films (a) upon heating, and (b) upon cooling.

Figure 9: X-ray diffraction patterns at different temperatures for: non-neutralized film and for neutralized film. Right figures show results of fitting (see text). *T, L-2* and *An* referred to the crystalline phases; *AM* indicates the amorphous fraction.

Figure 10: Schematic of the structure of neutralized (a, c) and non-neutralized (b, d) chitosan at the temperatures below glass transition (a, b) and above glass transition temperature (c, d).

Graphical Abstract

Temperature-dependence relaxation time of OH side groups *versus 1/T* for chitosan films in the heating and cooling process. Solid lines represent Arrhenius-type dependence fitting. Schematic of proposed of molecular interactions.