ISSN 1062-8738, Bulletin of the Russian Academy of Sciences: Physics, 2023, Vol. 87, Suppl. 1, pp. S66–S70. © The Author(s), 2023. This article is an open access publication, corrected publication 2024.
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Brillouin Scattering Study of Gelatin Films with Different Water Concentrations

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Received September 14, 2023; revised October 2, 2023; accepted October 9, 2023

Abstract—The gigahertz longitudinal elastic modulus of hydrated gelatin films as a function of water content was studied using Brillouin spectroscopy. It was found that the elastic modulus increases with increasing protein concentration. Two ranges can be distinguished with different concentration behavior, which are associated with different proportions of bonded water molecules. The addition of glutaraldehyde has very little effect on the elastic modulus of hydrated gelatin films.

Keywords: Brillouin spectroscopy, longitudinal elastic modulus, medical gelatin, gelatin films, glutaraldehyde stabilizing agent, two-phase model

DOI: 10.3103/S1062873823704427

INTRODUCTION

Hydrogels based on biopolymers such as medical gelatin are used as model systems to mimic tissues and have various applications in biomedical fields. The properties of hydrogels, which depends on the composition and method of preparation, must be optimized for a particular purpose. One of the important parameters is the mechanical elastic modulus. Mechanical techniques such as atomic force microscopy (AFM) [1, 2] and rheological mechanical testing [3, 4] are widely used to measure Young's modulus and deformability of hydrogels. The ability of Brillouin spectroscopy to characterize the viscoelastic properties on a scale of hundred nanometers has recently contributed to its development and application to biological and biomedical objects [5–8]. This rapidly developing area poses several challenges for researchers.

A complicating factor in the study of tissues and hydrogels is water, which changes the elastic response of biomolecules due to hydration and in the same time contributes to the overall response as a material fraction that has its own longitudinal modulus. Recent Brillouin studies evidence that the gigahertz elastic response of hydrated tissues and biomolecules is strongly affected by water content [9, 10]. These works presented different descriptions and interpretations of the influence of water on the longitudinal modulus. Therefore, further studies for this problem are currently relevant.

Cross-linking (stabilizing) agents such as glutaraldehyde are applied for tissues and hydrogels for various biomedical purposes. These agents crosslink protein molecules with intermolecular bonds and, as a result, can change the mechanical properties of the material [11]. It would be important to describe the effect of stabilizing agents on mechanical properties in the GHz frequency range using Brillouin spectroscopy.

In this work, Brillouin spectroscopy is applied to medical gelatin hydrogels with different water content and treatment with glutaraldehyde to describe the behavior of the longitudinal elastic modulus.

EXPERIMENTAL

Hydrogel Preparation

Commercially available partially hydrolyzed collagen (medical bovine gelatin) was used without further purification. Gelatin powder was mixed with distilled water in a ratio of 1 : 5 by weight. The mixture was kept on a water bath at 55°C until a homogeneous solution was obtained. To form the hydrogel, 30 mg of the aqueous gelatin solution was placed on a glass substrate at room temperature 20°C to complete gelation. Thus, hydrogel films with a diameter of 6 mm and a thickness of 1 mm were obtained. Gelatin films were placed in a sealed ampoule with a fixed humidity and stored for 5 days. Humidity was varied using aqueous solutions of glycerol of various concentrations. This allowed us to obtain films with various water content in hydrogel. Part of the sample was used to determine the protein concentration in the films by drying methods. The protein concentration C_g was calculated as $C_g = m_{dry}/m_{hyd}$, where m_{dry} is the weight of the dried sample and m_{hyd} is the weight of the hydrated sample.

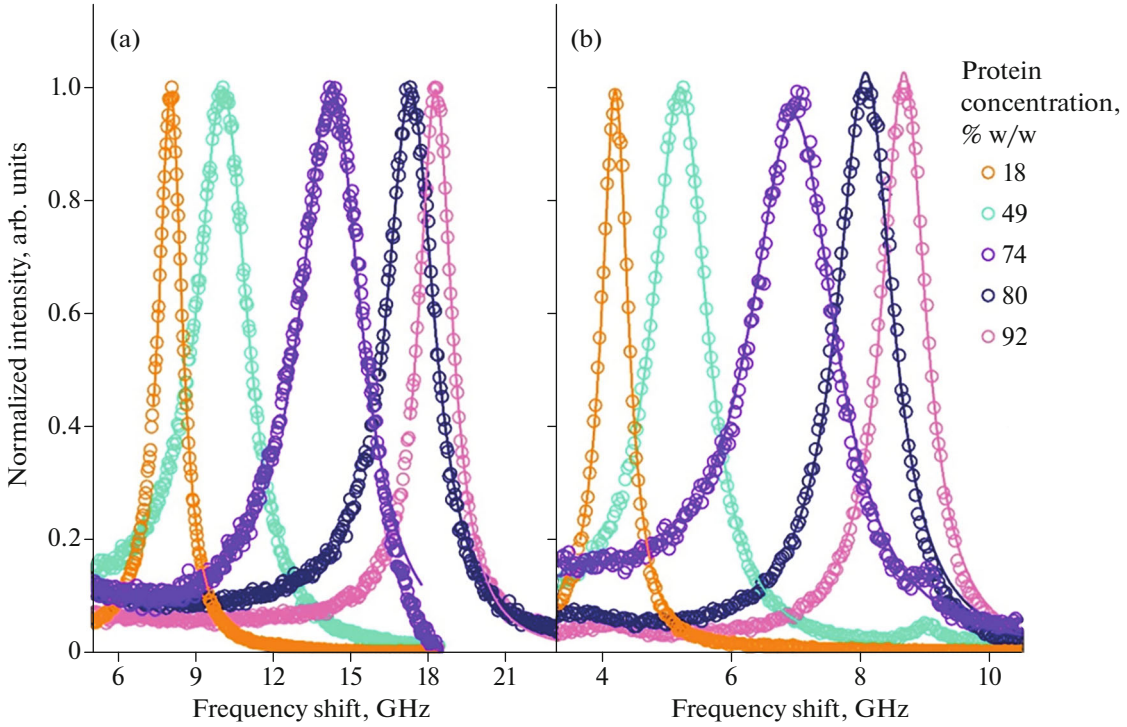

Fig. 1. Brillouin spectra of hydrated gelatin films for geometries BS (a) and 90A (b) (symbols). The solid line are the fits by the DHO function, Eq. (1).

Glutaraldehyde Treatment

Treatment of medical gelatin with glutaraldehyde (GA) was carried out by placing hydrogel films horizontally in a sealed cuvette over vapors of a 25% GA aqueous solution. The change in the concentration of crosslinking agent in the hydrogel was realized by changing the exposure time in GA vapor. The exposure time varied from 1 to 48 h. After that, the sample was placed in a hermetic ampoule with 100% humidity to maintain its hydration.

Determination of GA fraction

The GA molecules in the films cause them to turn yellow. The more GA molecules in a sample, the more yellow it is. The change of GA molecules embedded in the film was determined from the change in the optical absorption of the samples. The absorption spectrum of the crosslinked gelatin films was measured after drying using a Shimadzu UV-3100 spectrometer. Absorbance at $\lambda = 436$ nm normalized to the sample thicknesses was used for analysis.

Brillouin Experiment

Brillouin scattering spectra were measured using a tandem Fabry–Perót interferometer (JRS Scientific Instruments) and laser radiation at a wavelength of 532 nm. The free spectral range varied from 18.5 to 34 GHz depending on the position of the Brillouin line. The finesse was 100, which provided a spectral resolution of 0.3 GHz. Two orientations of planar samples with respect to the incident and collected light were applied. The backscattering (BS) geometry provided a scattering vector perpendicular to the plane of the hydrogel film. Another orientation (the so-called 90A) corresponded to the scattering vector along the film. The 90A and BS geometries allowed us to determine the sound velocity along and perpendicular the film, respectively.

RESULTS AND DISCUSSION

Brillouin Spectra

The Brillouin spectra of hydrated gelatin films in BS and 90A scattering geometries are shown in Fig. 1 for representative concentrations. The peak in the spectra is observed at the frequency of acoustic phonons satisfying to the wavevector selection rule. The Brillouin peak shifts to higher frequencies with increasing gelatin concentration for both geometries. Also, for some concentrations, the width of the Brillouin peak increases. This is attributed to a change in the relaxation response of the material, which depends on the water content. The largest line width corresponds to the water concentration, when the maxima of the relaxation susceptibility and the Brillouin peak are at the same frequency.

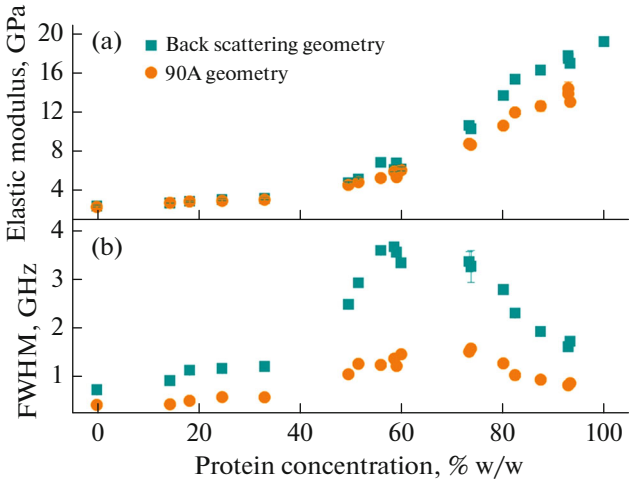

Fig. 2. Concentration dependences of the elastic modulus (a) and Brillouin peak width (b) for BS (blue squares) and 90A (orange circles) scattering geometries.

The Brillouin peak parameters were determined from the experimental spectra using the damped harmonic oscillator (DHO) function:

$$I(\omega) = \frac{I_0}{\pi} \frac{G_0 \omega_0^2}{\left(\omega^2 - \omega_0^2\right)^2 + (G_0\omega)^2}, \quad (1)$$

where ω_0 is the frequency shift of Brillouin peak and G_0 is the line width. Examples of DHO fits are shown in Fig. 1.

Elastic Modulus

For the case of BS geometry, the Brillouin frequency ω_0 is related to the sound velocity v_{BS} of phonons propagating along the film via:

$$v_{BS} = \frac{\Delta\omega\lambda_0}{2n}. \quad (2)$$

For the 90A scattering geometry, the Brillouin frequency ω_0 is linked to the sound velocity v_{90A} of phonons propagating across the film via:

$$v_{90A} = \frac{\Delta\omega\lambda_0}{\sqrt{2}}. \quad (3)$$

In Eqs. (2), (3), λ_0 is the wavelength of light and n is the refractive index. The refractive index n for Eq. (2) was calculated as $n = 0.00217C_g + 1.331$, according to [5].

The sound velocities found from the Brillouin spectra with Eqs. (2), (3) were used to calculate the longitudinal elastic modulus $M' = \rho v_s^2$. Film density ρ was found as $\rho = \frac{\rho_g \rho_w}{C_g(\rho_w - \rho_g) + \rho_g}$, where $\rho_g = 1.345$ g/cm^3 is the density of dry gelatin, $\rho_w = 1$ g/cm^3 is the water density [5]. The found values of M' and the full width at half-maximum (FWHM) of Brillouin peaks are shown in Fig. 2.

The concentration dependences of M' for phonons propagating along and across the film are shown in Fig. 2a. The elastic modulus increases with increasing gelatin concentration for both types of phonons. Close values of the modulus for both directions of phonon propagation indicate that the hydrogel films are elastically isotropic. Some difference in M' for $C_g > 60\%$ can be explained by the frequency dispersion of the relaxation susceptibility. The concentration dependence of FWHM (Fig. 2b) allows us to conclude that, at $C_g > 60\%$, the maximum of the relaxation susceptibility is at frequencies lower than the Brillouin peak frequency.

Two ranges can be distinguished on the dependences $M'(C_g)$ (0–60 and 60–100%, Fig. 2a), which are different in the slope. This observation points a different role of water concentration for these ranges. In hydrogels, some water molecules have strong hydrogen bonds with protein molecules (bound water) and some have the properties of pure water (bulk water), forming water-filled pores. There are estimates in the literature that bulk water disappears in protein solutions at $C_g > 60$–70%. Thus, the range of C_g from 0 to 60% in Fig. 2a can be associated with the presence of bulk water, which contribute significantly to M'. The elastic modulus in the range above 60% is determined by hydrated protein molecules whose mechanical response varies greatly with C_g.

Two-Phase Model of Elastic Modulus

At the concentration $C_g < 60$–70%, the hydrogel film contains two components: water-filled pores (bulk water) and hydrated protein molecules, which contribute to the elastic modulus. We took into account these two components using two-phase layered models. It was found that the model that summing the elastic compliances describes well the experimental data. This case corresponds to the condition of equal stresses for the components, and the elastic modulus is described by:

$$\frac{1}{M} = \frac{f}{M_g} + \frac{1-f}{M_w}, \quad (4)$$

where $f = \frac{V_g}{V_g + V_w}$ is the volume fraction of dissolved solids (V_g, V_w are the volumes of gelatine and water, respectively), M_g is the elastic modulus of the dissolved solid (gelatin), M_w is the elastic modulus of the solvent (water).

The comparison of the elastic modulus from the Brillouin experiment and the two-phase model is shown in Fig. 3. It is seen that the concentration

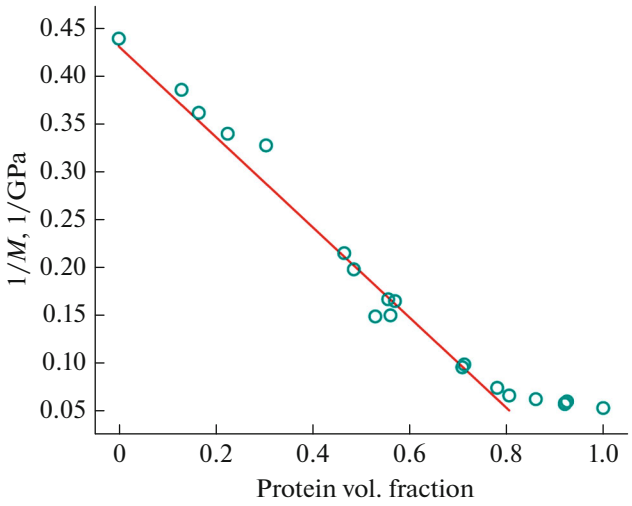

Fig. 3. Elastic compliance of the hydrogel versus the volume concentration of protein. The solid line is a linear function.

dependence of the elastic compliance $1/M'(C_g)$ (data of BS geometry are used in this figure) is a linear function at $C_g < 70\%$ as expected from Eq. (4). Thus, the elastic modulus of the gelatin hydrogel is well described by the model in which the compliance of bulk water and hydrated protein molecules contribute additively.

The concentration limit of applicability of the two-phase model is about 70–80%. At this concentration, all water molecules participate in protein hydration. A further decrease in water concentration decreases the hydration of proteins and makes their elastic response more rigid.

GTA Treatment

Figure 4a shows the results for Brillouin position (the BS geometry) versus protein concentration, including films treated with glutaraldehyde (GA). It is seen that the GA treatment increases the scatter of the values, which may be due to local inhomogeneities in the GA concentration. Comparing with the results for hydrated untreated samples (shown as green squares in Fig. 4a), it can be concluded that there is the effect of crosslinking agents on the gigahertz elastic modulus is weak compared to the water-induced effect.

Considering the effect of the GA treatment on the polymer matrix solely, we studied the Brillouin spectra of dried samples. Figure 4b shows the dependence of the Brillouin shift on GA concentration, quantified in terms of absorbance at 436 nm. The GA concentration is proportional to the absorbance at 436 nm. It is seen (Fig. 4b) that the Brillouin frequency decreases with increasing GA concentration. This decrease is caused by GA molecules preventing the compact protein structure after drying. In the structure of dried gelatin,

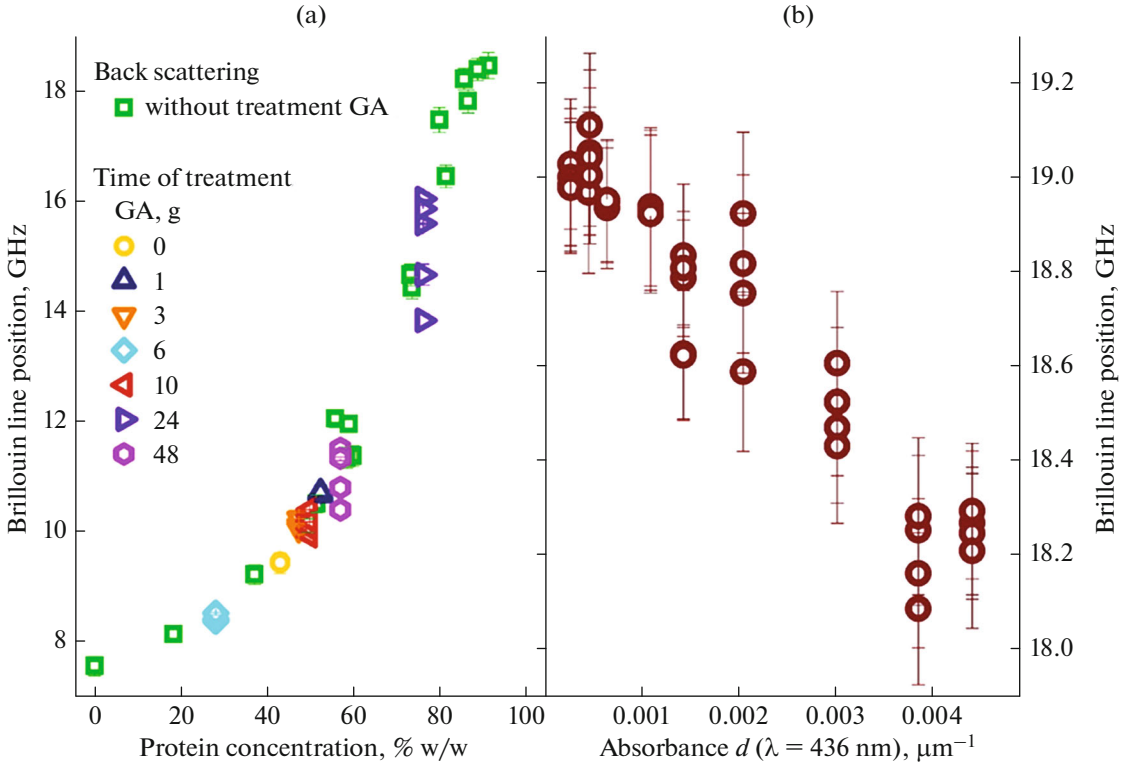

Fig. 4. (a) Brillouin shift versus protein concentration for GA treated (colored symbols) and untreated (green squares) gelatin films. (b) Brillouin shift of dried hydrogels versus GA concentration, quantified by absorbance at 436 nm.

cross-linking GA molecules play the role of structural defects. The less compact the structure of dried gelatin, the lower the elastic modulus of gelatin films is expected.

CONCLUSIONS

The Brillouin spectroscopy was used to study the longitudinal elastic modulus of hydrated films based on medical gelatin at gigahertz frequencies. The results for two Brillouin scattering geometries indicate the isotropy of the film investigated. The longitudinal modulus increases with increasing protein concentration. In the range of protein concentration from 0 to 60–70%, the increase in the elastic modulus is well described by an additive model that combines the contributions from bulk water and hydrated protein. The concentration dependence in the range from 60–70 to 100% corresponds to the absence of bulk water, and the elastic modulus is governed by changes in protein hydration. Decreased hydration of proteins makes them more rigid. We found that glutaraldehyde treatment had a smaller effect on the elastic modulus compared to hydration. The effect of this cross-linking agent was observed in Brillouin experiment with dried gelatin films, for which glutaraldehyde plays the role of structural defects.

ACKNOWLEDGMENTS

The experiments were partially performed using the equipment of the Center for Collective Use "High-Resolution Spectroscopy of Gases and Condensed Matter" of the Institute of Automation and Electrometry, Siberian Branch, Russian Academy of Sciences (Novosibirsk, Russia).

FUNDING

This work was supported by the RF Ministry of Science and Higher Education, grant no. 121032400052-6.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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