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ABSTRACT

Analysis based on the determination of the multifactorial non-linearity parameter (β) is a promising non-destructive investigation and testing technique. The contribution of temperature variations on the non-linear coefficient is known to be lower than that of hydrostatic pressure changes. We investigated the effect of temperature on the non-linearity parameter in the range 30 °C–60 °C for a viscous, gelatinous compound, resulting from controlled hydrolysis of the collagen protein. Considerable thermal effects are realized and are related to changes in viscous and elastic properties. Remarkable changes in the non-linearity coefficient at temperatures corresponding to the transition temperature of gelatine of 60 °C indicate a signature while no outspoken hysteresis effects were realized with cyclic temperature sweeps. Despite the non-Newtonian nature of the gel, our experiments show comparability to water within the examined range of temperature, which corresponds to a wavelength shift of about 40 μm .

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I. INTRODUCTION

Gelatine is a viscous biomaterial known to be versatile and multi-functional hydrocolloid. It is a hydrolyzed form of the most abundant protein, collagen, which can be processed into either an acidic type or alkaline type.¹ It is often utilized in the industry of various kinds of food products^{2,3} as a gelling, clarifying, foaming, or emulsifying agent, among other functions.⁴ It is also used in the pharmaceutical industry as soft or hard microspheres.⁵ The proper way to dissolve gelatine powder in water is to mix the powder in hot, but not boiling, water that is at least at a temperature of 55 °C.⁶ Techniques such as differential scanning calorimetry (DSC) and static light scattering have been used to investigate the effects of temperature on the thermoreversibility and kinetics of gels.⁷ In the current research, a trade gelatine meant for food consumption is characterized by studying its ultrasonic non-linearity coefficient and its viscoelastic properties over a range of temperature change. The thermally reversible gelling capability of gelatine is tested by repeating the cooling process by reheating the material.

Investigation and evaluation of the thermoreversible properties of gels are useful in assessing the suitability and compatibility of

gelatine alternatives, which are frequently in demand for a broader variability in food production.

Ultrasonic non-linearity is an intrinsic property that originates from the interatomic and intermolecular potentials and is governed by many factors, mainly the variations in temperature, pressure, and density in addition to ultrasonic parameters such as the pathlength and thermal and viscous losses of the transmitted signal. The non-linearity coefficient (β) is, therefore, a characteristic parameter that is useful in characterizing these parameters in a material.⁸

A widely accepted model for non-linear acoustic propagation is based on a perturbation procedure, as expressed in many references such as that by Bjørnø,⁹ where the variables in the equations of continuity, of motions, and of energy were replaced by the sum of their equilibrium values [variables with a subscript (0) in the following relations]¹⁰ and their first and second order variation components,¹⁰

$$\rho = \rho_{(0)} + \rho_{(1)} + \rho_{(2)} + \dots, \quad (1)$$

$$T = T_{(0)} + T_{(1)} + T_{(2)} + \dots, \quad (2)$$

$$p(\rho, T) = p_{(0)}(\rho_{(0)}, T_{(0)}) + \left[\left(\frac{\partial p}{\partial \rho} \right)_T \right]_{(0)} (\rho - \rho_{(0)}) + \left[\left(\frac{\partial p}{\partial T} \right)_\rho \right]_{(0)} (T - T_{(0)}) + \dots, \quad (3)$$

where p is the pressure and T is the temperature. The pressure can be further expressed in the thermodynamic equation of state, considering the instantaneous variations in the entropy.

Since propagation of ultrasound is essentially isentropic, where the static value of the entropy can be assumed to be equal to the instantaneous entropy, the isentropic equation of state is expressed as^{11,12}

$$\rho - \rho_{(0)} = A \left[\frac{(\rho - \rho_{(0)})}{\rho_{(0)}} \right] + \left(\frac{B}{2} \right) \left[\frac{(\rho - \rho_{(0)})}{\rho_{(0)}} \right]^2 + \frac{C}{6 \left[\frac{(\rho - \rho_{(0)})}{\rho_{(0)}} \right]^3} + \dots, \quad (4)$$

where the coefficients A , B , and C are defined as

$$A = \rho_0 \left[\left(\frac{\partial p}{\partial \rho} \right)_0 \right]_{\rho=\rho_{(0)}} = \rho_0 c_0^2, \quad (5)$$

$$B = \left[\rho_0^2 \left(\frac{\partial^2 p}{\partial \rho^2} \right)_s \right]_{\rho=\rho_{(0)}}, \quad (6)$$

$$C = \left[\rho_0^3 \left(\frac{\partial^3 p}{\partial \rho^3} \right)_s \right]_{\rho=\rho_{(0)}}, \quad (7)$$

where c_0 is the velocity of propagation of ultrasonic waves of infinitesimal amplitude.

With rearrangements and substitutions, the second order non-linearity coefficient B/A can then be expressed as¹⁰

$$\frac{B}{A} = 2\rho_0 c_0 \left\{ \left(\frac{\partial c}{\partial p} \right)_T \right\}_{\rho=\text{const}} + (2c_0 T \gamma c_p^{-1}) \left\{ \left(\frac{\partial c}{\partial T} \right)_p \right\}_{\rho=\text{const}}, \quad (8)$$

where γ is the volume coefficient of thermal expansion. It is clear in relation (4) that the dominant factors are temperature and pressure. The non-linear dependence of the measurable ultrasonic parameters is easily detectable upon subjection of the sample to finite amplitude ultrasonic waves,¹³ which is similar to subjection to stress or other types of perturbation that results in an elastic reaction of measurable amplitude.

In the current study, ultrasound is applied at variable temperatures, and the corresponding variations in the signal amplitude are used to deduce the relative non-linearity coefficient.^{14,15} The variations in the velocity of propagation of ultrasonic waves are also investigated in addition to the variation in the viscous and thermal dissipations of the ultrasonic signal due to changes in temperature, since it is directly related to the bulk modulus of rigidity. This is similar to works carried out to determine the temperature effects on the non-linearity

parameter for water by Maraghechi *et al.*,¹⁶ in animal livers by Zhang,¹⁷ and in silica gel formation by Robin *et al.*¹⁸

The motivation toward characterizing gelatine stems from the fact that it is a trade material manufactured in bulk quantities, so a non-destructive contribution to quality control and health monitoring is expected to meet the interests of a great deal of institutions and a larger community. Another motivation comes from the fact that upon exposure to temperatures higher than room temperature, gelatine experiences a phase-transition.¹⁹ We anticipate our facilities and the ultrasonic non-linear techniques to be a feasible way to monitor structural changes during the transition and that these changes are reversible, providing an additional technique to the state of the art so far.

II. MATERIALS AND METHODS

An off the shelf, nutrient-free, sugar-free, ready to use (Backfee, made in Germany) gelatine powder was purchased and used for this investigation. The recommended dry concentration for a 500 ml solvent is 9 g (one packet), mixed and filtered to obtain a jelly-like soft solid compound.

The gelatine liquid solution is prepared with 63.00 g of Backfee gelatine powder. The powder is then dissolved in 700 ml reverse osmosis filtered tap water at a temperature of 70 °C. The mixture is stirred until all the powder is completely dissolved in the liquid.

The temperature at which gelation takes place is variable depending on the average molecular weight, ionic strength, acidity, cooling rate, and method of determination.²⁰ Likewise, the solid-liquid phase transition could be variable.²¹ The sample of gelatine investigated in this research was found to exhibit a phase transition at a temperature of 60 °C.

Once the powder was completely dissolved, the mixture was poured into an aluminum saucepan with holes, allowing identical emitter and receiver transducers aligned concentrically. The faces of the emitter and receiver transducers are separated by 54 mm. Both the emitter and receiver transducers were completely submerged in the mixture. Measurements were performed, while the temperature was recorded simultaneously. The temperature was cycled from 60 °C to room temperature and vice versa to test the reversibility. Water was also measured as a reference sample.

A piezoelectric transducer with a resonance frequency of 1 MHz and a diameter of 12.7 mm is used to emit mechanical pressure waves that propagate through the gel material and is received by the identical receiver transducer. Generation and detection of the burst signal are carried out by a RITEC Advanced Measurement System (RAM-5000), which allows controlling the different parameters, including the power density, repetition rate, number of cycles, frequency, and amplitude. A burst with a 20 cycle sinusoidal signal with a resonant frequency of 1 MHz was applied in all experiments. A LeCroy oscilloscope (64Xi) is used to record the calls sent to the transmitter and those received by the receiving transducer. The signal profiles were recorded at a sampling frequency of 25 MHz. An Omega HH800 thermocouple is used to record the temperature of the liquid-solid samples. The temperatures are sampled at a sampling frequency of 1 Hz. The temporal spectra and temperature change over time were recorded simultaneously. A simple schematic of the experimental setup is shown in Fig. 1.

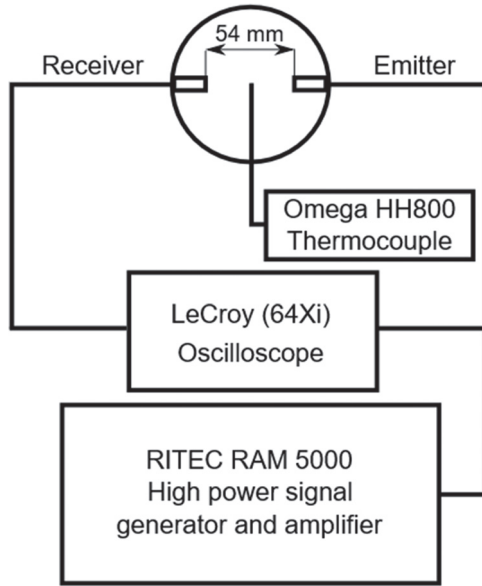


FIG. 1. Schematic of the experimental setup.

III. RESULTS AND DISCUSSION

The measurements were carried out and the temperature was recorded while the liquid cooled from around 60 °C to below 30 °C. To investigate any possible hysteresis effects, the solution was again heated up to above 60 °C and cooled down to below 30 °C while taking regular measurements during the process.

The speed of sound was calculated by comparing the time of arrival of the first peak of the receiving transducer signal to the time of arrival of the first peak of the signal to the transmitting transducer (time of flight),

$$c = \frac{d}{t_{received} - t_{transmitted}}, \quad (9)$$

where d is the distance between the transducer faces (a constant 54 mm for this experiment) and $t_{received}$ and $t_{transmitted}$ are the time of the first peaks in the receiving and transmitting transducer signals, respectively.

The degree of non-linearity for a pressure-wave propagating within the material can be modeled using the Khokhlov-Zabolotskaya-Kuznetsov (KZK) equation and parabolic approximations,²² with scattering expected to be negligible and attenuation only due to absorption (viscous and thermal) and minor reflections,

$$p^{rec} = p^{inc} + p^{refl}, \quad (10)$$

$$p^{inc} = \frac{-jk_2^2 S \beta (p^{emit})^2 R_1}{8\pi \rho c^2} e^{-2\alpha f^2 h} e^{-2jk_2 f} F^{inc}, \quad (11)$$

$$p^{refl} = \frac{-jk_2^2 S \beta (p^{emit} R_1)^2}{8\pi \rho c^2} e^{-2\alpha f^2 h} e^{-2jk_1 h} e^{-\alpha(2f)^2 h} e^{-jk_2 h} F^{refl}, \quad (12)$$

where k_1 and k_2 are the wave numbers for the emitted and reflected waves, respectively, α is the attenuation coefficient, F^{inc} and F^{refl} are

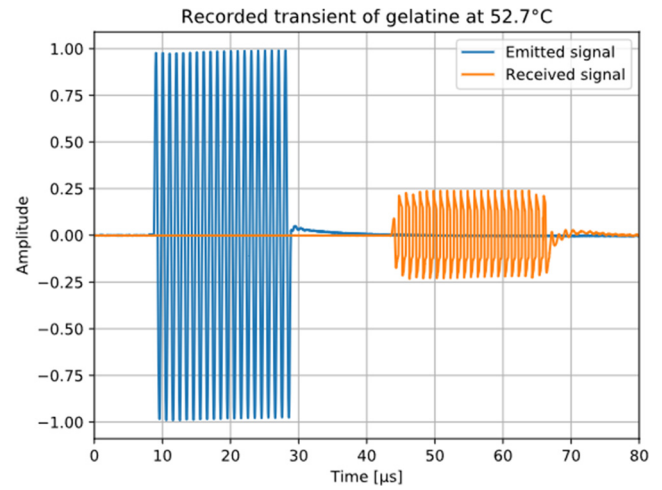


FIG. 2. Recorded temporal signal for gelatine at 52.7 °C.

the diffraction factors expressed as sums of the Gaussian beams, R_1 is a reflection coefficient, ρ is the density of the medium, c is the speed of sound in the medium, f is the frequency of the emitted wave, S is the surface area of the transducer, h is the distance between the transducers, and β is the non-linear parameter.

The non-linearity coefficient (β) is calculated using the maximum amplitude of the fundamental frequency (A) and the maximum amplitude of the first higher harmonics (B), based on the ratio in Eq. (8).²² The amplitudes are calculated from the FFT of the temporal signals:

$$\beta = 1 + \frac{A}{2B}. \quad (13)$$

A sample of the recorded temporal signal and its FFT magnitude for gelatine at 52.7 °C are shown in Figs. 2 and 3, respectively.

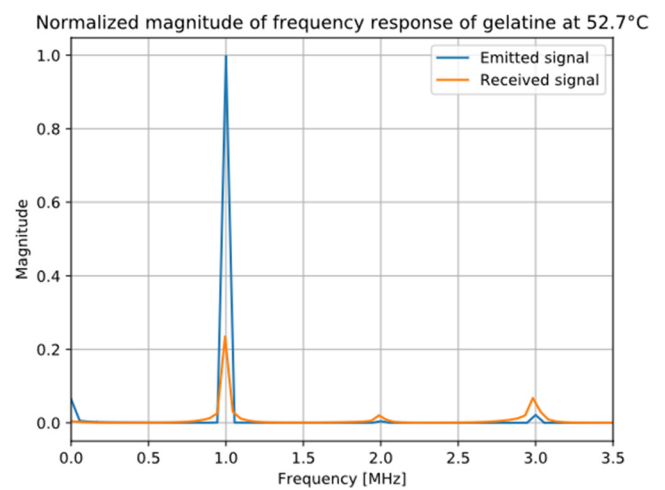


FIG. 3. Magnitude of the frequency response of the temporal signal of gelatine at 52.7 °C.

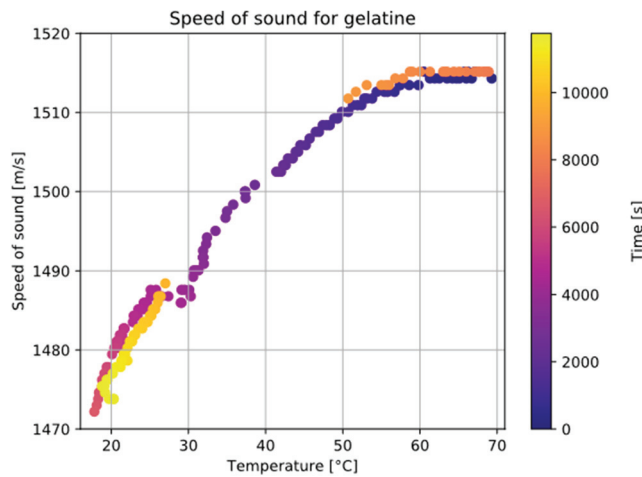


FIG. 4. Velocity of ultrasound in gelatine for variable temperatures. The color map represents the time scale in seconds.

The velocity of propagation of ultrasound in gelatine for the investigated temperature range is shown in Fig. 4. The curve of the speed of ultrasound in water is depicted in Fig. 5 for comparison. A higher propagation velocity in gelatine is realized, with similarity to the speed vs temperature of pure water. Possible hysteresis effects were also investigated to determine if the effects observed would be the same if the temperature cycle is repeated by reheating and cooling again.

Some minor temperature lag artifacts appear in these results since the temperature changes are not perfectly uniform throughout the entire material.

The representation of the non-linear coefficient for gelatine with variable temperatures is depicted in Fig. 6, and that for water is shown in Fig. 7. The abrupt change in the values of β at about 60°C can be attributed to the phase transition known to occur around this

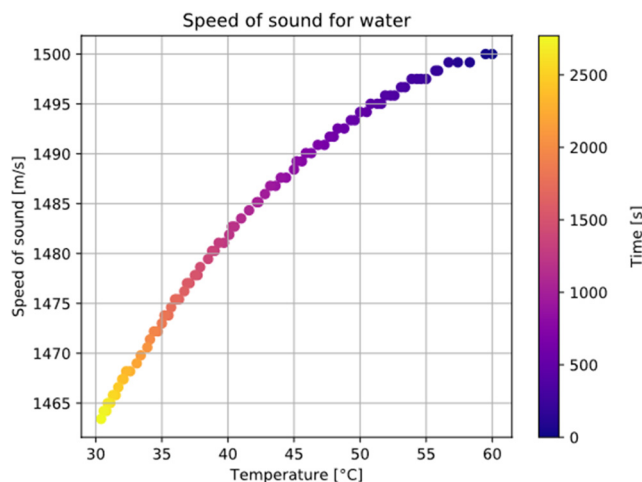


FIG. 5. Velocity of ultrasound in water for variable temperatures. The color map represents the time scale in seconds.

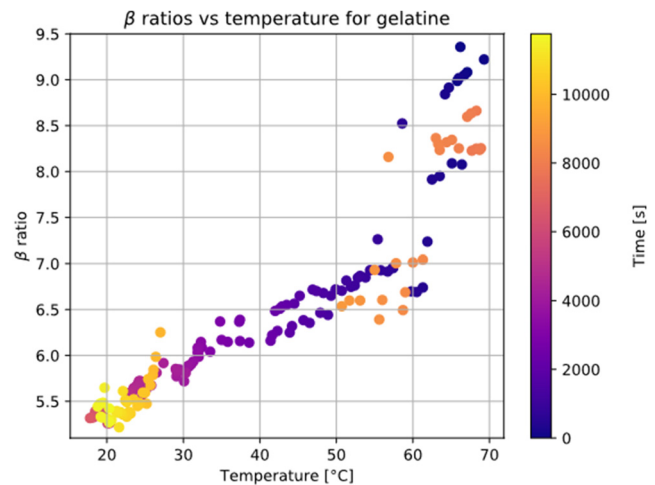


FIG. 6. Non-linear coefficient vs temperature for gelatine. The color map represents the time scale in seconds.

temperature. Although, roughly speaking, hysteresis effects do not appear, there are indications that perhaps minor effects can be caused by cycling the temperature. Indeed, minor deviations in the values of β , as the temperature is cycled, are observed and may possibly indicate variations in the molecular structure or in the compressibility. In addition to thermal effects on the molecular structure, hydrostatic pressure is also involved. Experiments based on differential scanning calorimetry (DSC) applied under ambient pressure and light-transmittance measurements under high pressure indicated a considerable effect of pressure changes on mixed-chain lipid phase transition.²³ Temperature and pressure dependence of phase transition result in high sensitivity of ultrasonic non-linearity to the sol-gel and gel-sol transition, which can serve in tuning of the gel properties in the various applications that involve gelatin, among other applications.

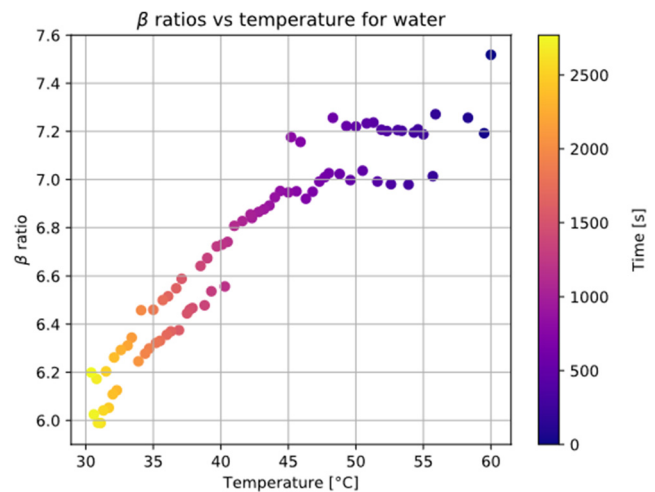


FIG. 7. Non-linear coefficient vs temperature for water. The color map represents the time scale in seconds. For interpretation, note the narrow range of β in comparison with Fig. 6.

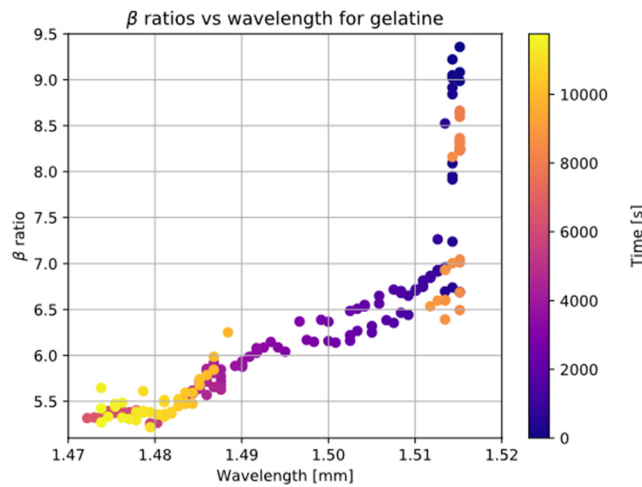


FIG. 8. Non-linear coefficient vs wavelength for gelatine. The color map represents the time scale in seconds.

It is worth noting that despite our concern with the effects of heating and cooling rates,²⁴ the cooling in these experiments was spontaneous cooling at room temperature to allow a natural evolution of the transition during temperature sweeps. The heating rate was, however, about 2 °C/min.

The non-linear parameter for gelatine as a function of wavelength is shown in Fig. 8. A wavelength shift of about 40 μm is observed for the investigated temperature range, which resembles a basis on which acoustic spectral monitoring of the local phase transition can be routinely studied. The variation in the values of β of the gelatine measured during warming after cooling may indicate a loss in the strength of coupling in the dynamics of the gel network and those of the solvent liquid.²⁵

The β ratio as a function of wavelength for filtered tap water is also shown in Fig. 9, where a comparable shift in wavelength is realizable.

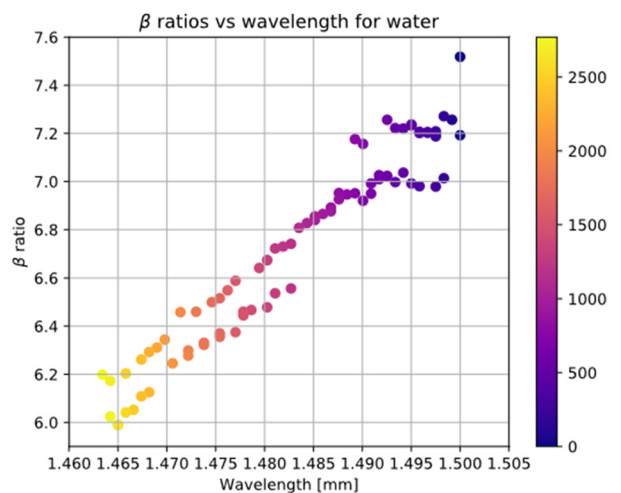


FIG. 9. Non-linear coefficient vs wavelength for water. The color map represents the time scale in seconds.

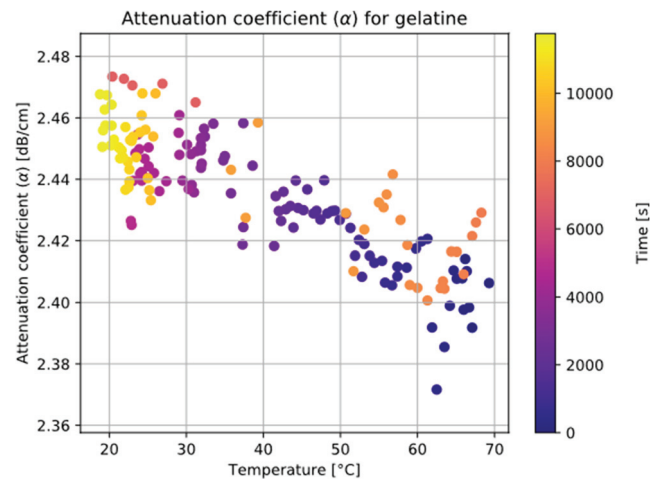


FIG. 10. Normalized attenuation coefficient vs temperature for gelatine. The color map represents the time scale in seconds.

Considering the non-linearity parameter against wavelength allowed refining some of the temperature lag effects. A sharp transition could indicate a rapid change in the ratio of the storage and loss moduli.²⁶

The attenuation coefficient of gelatine is also compared to water over the same temperature range. The attenuation coefficient (measured in dB) is defined as follows:

$$\alpha = 10 \log_{10} \left(\frac{A}{A_0} \right)^2, \quad (14)$$

where A is the received amplitude and A_0 is the generated amplitude. The attenuation coefficient is normalized over the distance between the transducers in centimeters to produce the results shown in Fig. 10 for gelatine and Fig. 11 for water.

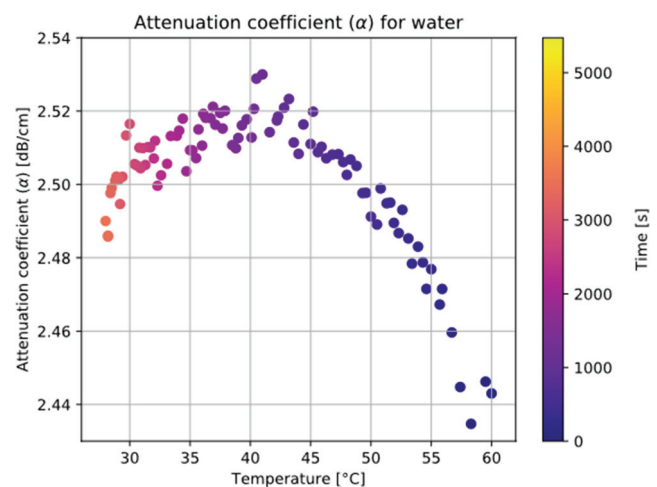


FIG. 11. Normalized attenuation coefficient vs temperature for water. The color map represents the time scale in seconds.

The main constituent protein of gelatine, collagen, is subjected to denaturation during the preparation phase upon subjection to hot water. On cooling, relaxation takes place, and the polymer fragments are re-constructed but not necessarily fully recovered to the initial native collagen structure.²⁷ In light of a minor observed hysteresis effect, it is expected that the degree of reversibility depends on the degree of denaturation and renaturation recovery. The incomplete renaturation is realized as shown in Figs. 6 and 8, where the values of β are not completely repeated for the same temperature after repeating the temperature cycle. The secondary skeleton of gelatine is made up of hydrogen bonds,²⁸ which form intermolecular weak forces; therefore, the gel is expected to be reversible upon dissociation under the effect of temperature, or else, there could be some degree of inhomogeneity.

IV. CONCLUSIONS

The measured speed of sound for this investigation of gelatine and its temperature dependence showed a similar profile to well-known results for the effects of temperature on the speed of sound in water. The sensitivity of the measurement of the non-linearity coefficient to temperature can be used to investigate structural changes in the gel.

A distinctive sharp change in the trend of the values for gelatine is evident, which indicates phase transition at 60 °C.

No clear evidence for significant temperature hysteresis effects was found while reheating and cooling the gelatine mixture. Nevertheless, minor deviations in the values of non-linearity, when the temperature cycle is repeated, possibly indicate minor variations in the molecular structure due to the degree of denaturation and renaturation of the collagen protein. Apart from the detected minor irreversibility, we may conclude that the effect of temperature on the non-linear wave profile in gelatine is to a very large extent reversible.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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