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Ultrasonic Velocity and Absorption Measurements in Egg White

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Abstract

Ultrasonic measurements are made in egg white to study the properties of the solution of the natural protein. The high-Q ultrasonic resonator method is used to get the ultrasonic absorption spectra over the range 0.2-10 MHz at 20℃. It is proportional to the 1.25th power of the frequency. The gelation process caused by heat is studied from the change in the velocity and the absorption. at 3 MHz using the pulse echo overlap technique over the range of 10-80℃. The absorption decreases with increasing temperature up to 60℃ where it turns up sharply and rapidly increases thereafter. The strong absorption in the gel region is described by the interaction between the solution and the network structure made of protein. Very slow variation in time elapse is observed after the temperature is quickly raised. It would be a real-time observation of the network building process and the characteristic time for the process is shown to be 400 min. A hysteresis phenomenon with respect to the temperature is observed. This phenomenon is associated with the memorizing effect of the network structure of protein of the gel.

Keywords: *Egg white, Ultrasonic absorption, Ultrasonic velocity, Gelation*

I. Introduction

We have been interested in the ultrasonic spectroscopy for the purpose of studying the physical properties of materials, and have developed experimental techniques useful at various frequency range[1]. The high-Q ultrasonic resonator method, which was used in the present study, was developed to obtain the low-frequency spectrum in liquid and solutions. This technique has been successfully used to investigate the microscopic structure and the dynamical properties of material at molecular levels. Egg white has attracted attention of bioscientists as one of the typical substances of natural protein and some ultrasonic studies have been made by pulse technique[2]. In the present study, we measured the absorption and revealed the spectra over the range from 10 MHz to 0.2MHz at 20℃. What is more interesting is, however, the results at 3 MHz over a range of temperature. We found two notable phenomena. First, there appears an excess absorption over the normal curve of temperature dependence at the gelation transition point, when the specimen is slowly heated from raw egg to boiled egg. Second, a remarkable hysteresis effect is found with respect to the temperature in both the ultrasonic velocity and the absorption of egg white gel.

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II. Experiments

Ultrasonic resonator method is a standard technique to measure the ultrasonic absorption coefficient below 10 MHz, where the frequency is too low for the common pulse method or the interferometer to have sufficient accuracy[3]. The current construction of the resonator has two quartz transducers fixed face to face on both ends of a cylindrical spacer between which the liquid under study is put in. A sweep generator drives one of the transducer, which excites the cavity into resonance. The resonance spectrum is obtained by the other transducer. Width of one resonance peak gives the ultrasonic absorption in the liquid with a known value of instrumental width of the cavity. In this conventional resonator, the instrumental loss rapidly increases at lower frequencies and virtually inhibit the measurements below 1 MHz.

We have developed a high-Q ultrasonic resonator method. Briefly describing, one of the quartz transducers is replaced by a slightly concave reflector, which effectively suppresses the diffraction of the sound field and substantially reduces the instrumental width due to the diffraction loss particularly at frequencies below 1 MHz[1]. The resonance spectrum is optically detected by a laser which goes across the cavity so that Raman-Nath diffraction occurs.

Figure 1 shows the sectional view of the high-Q ultrasonic resonator used in the present work. The driving quartz is 60 *mm* in diameter and its fundamental frequency is 2 MHz. The concave reflector is made of stainless steel with a thickness of 1 *mm*. Its curvature radius is 400 *mm*. Circulating water regulates the temperature of the whole cavity with an accuracy of 0.5℃. Details of the experimental system have been described in the literature[1].

To study the temperature dependence and the effect of denaturation and gelation by heat, we made the absorption measurement at 3 MHz. The measurement system is essentially the pulse echo overlap technique with path length of 60 *mm*. This system is, however, partially modified to obtain the ultrasonic absorption as well as the velocity of the medium. Figure 2 shows the typical wave forms of the pulse and echo overlapped on CRT. The ratio of the two amplitudes is determined by a variable step attenuator which allows amplitude adjustment with a 0.1 dB accuracy. The wave form of the echo deforms by the reflection at the half wavelength quartz plates, and this deformation introduces apparent attenuation over the intrinsic absorption. The additional loss due to this effect is appropriately subtracted from the measured value in relative measurement made with water as a reference liquid. The acoustic impedance of the egg white is likely to equal with water. A calculation of the sound diffraction loss is also made by solving the equation of Schoch[4]

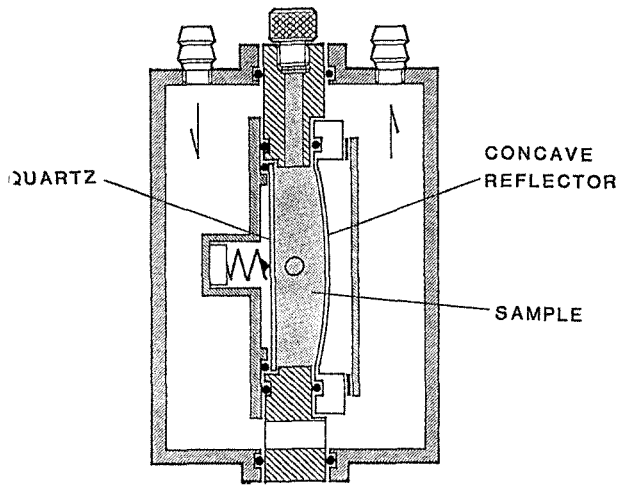

Figure 1. Sectional view of high-Q ultrasonic resonator. The circle in the center of the resonator is a window through which the probing laser pass. The arrows denote the water circulation.

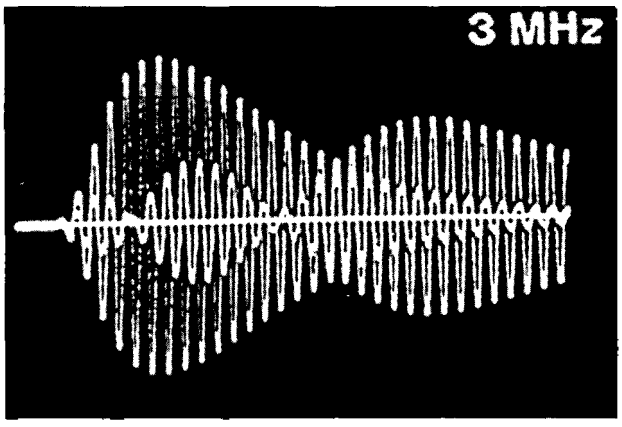

Figure 2. A typical example of pulsed and echoed signals overlapped on an oscilloscope. The ultrasonic frequency is 3 MHz.

Table 1. Concentrations of the thin portion of egg white[6].

Water	88%
Ovalbumin	5.4%
Conalbumin	1.2%
Ovomucoid	1.1%
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.	.

numerically with an effective radius (5 *mm*) of the source and f=3 MHz. The accuracy has been shown to be better than 10% for liquids with ultrasonic absorption coefficients higher than $\alpha/f^2 = 200 \times 10^{-15} s^2/m$. The triangle in Fig. 3 displays the result at 20℃ showing a very good agreement with the values by the resonator method (see the details given in section III). This system is very handy and convenient for simultaneous measurements of the velocity and the absorption, and suitable for the present experiment in which measurements should be made at various temperatures. The specimen is egg white taken from new laid eggs which have been kept at 5℃ for two days. Only the thin portion of the egg white was filtered through meshes and used as a sample after degassing by a vacuum pump. Table 1 shows the concentration of the thin portion of egg white which is, roughly, water solution of ovalbumin.

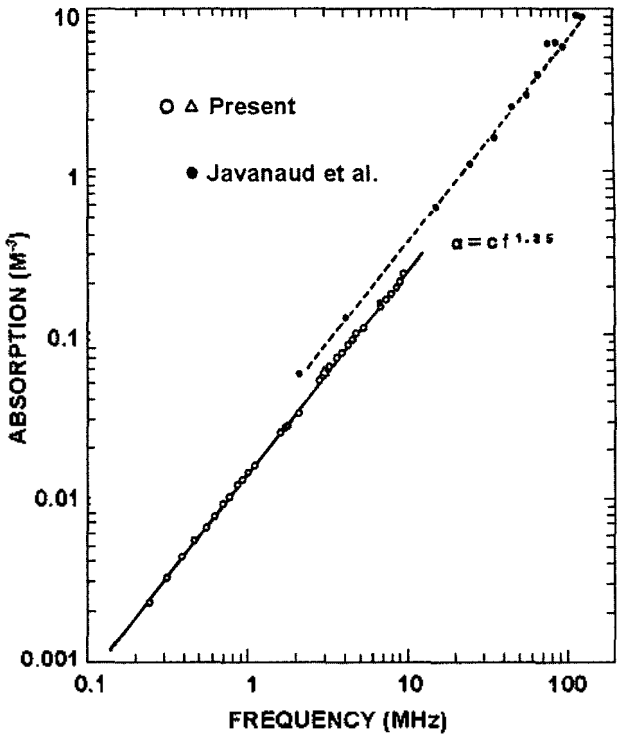

Figure 3. Ultrasonic absorption in egg white observed by high-Q ultrasonic resonator method over the range of 0.2-10 MHz. The triangle at 3 MHz was obtained by the pulse technique. The closed circles indicate the result by Javanaud et al[2]. The measured data are well fitted to the curve expressed by $\alpha=Cf^{1.25}$ as represented by the solid and dashed lines, though the value of C is different between the two works.

III. Results and Discussions

A. Absorption Spectra

Figure 3 shows the log-log presentation of the observed absorption coefficient versus frequency. The open circles and the triangle denote the present results at 20℃. Our experimental values are well fitted over the investigated frequency range to the equation

$$\alpha = Cf^{1.25\pm0.02},$$

which is indicated by the solid line in Fig. 3. Here the constant C is obtained to be 4.45×10^{-10} at 20℃ if α is expressed in m^{-1} and f in Hz. On the basis of the present work, it is concluded that absorption in egg white is proportional to the 1.25th power of frequency over the range of 0.2-10 MHz. Carstensen *et al.*[5] studied the ultrasonic absorption in hemoglobin and serum albumin solutions in the 0.8-3 MHz range and found that the absorption is proportional to the 1.2th power of frequency. The absorption coefficients including its frequency dependence are close to the present results obtained in egg white. This suggests that the mechanism of the relaxation occurring in egg white involving several kinds of proteins is essentially the same as that in a single protein solution such as albumin.

B. Denaturation and Gelation

The results obtained in the egg white are summarized in Fig. 4. The experiment started at 10℃ and the temperature was increased thereafter. The measurement at the temperature above 60℃ took a very long time since we should wait several hours after the sample was heated at

each temperature. Above the gelation temperature, the protein molecules in the egg white are denatured and aggregated to form a network structure[6], which causes the change in the properties of ultrasonic absorption and the velocity. The absorption and the velocity varied very slowly toward the saturation value as described later. The egg white sample is in sol state up to 60℃. Above 60℃ the denaturation and the gelation occur simultaneously. Network structure of protein polymer is gradually formed within the solution which turns opalescent and white to be a boiled egg. The network structure grows and gets closer as the temperature increases. Based on the analysis and discussion of the experimental results, we shall have a following description of egg white gel: egg gel is just like a loaf of sponge fully soaked in protein water solution.

The ultrasonic absorption coefficient at 3 MHz decreases with temperature until 60℃ of the gelation point, where it turns sharply upward. The overall profile may be described by superposition of two different mechanisms of absorption with different dependence on the temperature. The first one is the monotonous decrease appearing explicitly below 60℃, i.e., sol or raw region. This decrease is a natural trend common to every solution of protein as well as other molecules[7,8], and would perhaps continue well to above 60℃ in the solution within the gel. The second absorption mechanism arise from 60℃ and on. Absorption increases steeply with temperature and overwhelms the first mechanism. The probable cause for the second mechanism is the interaction between the formed network and the solvent. For example, friction loss may arise when the solvent goes to and fro through the network according to the ultrasonic cycle. The friction loss increases as the network gets finer. A quantitative analysis for this would be possible with an appropriate model of the drenched sponge. An alternative description of the absorption curve is that the gelation starts rapidly and immediately stops around the temperature of 60℃ and then starts again at 70℃. In contrast to the notable feature of the absorption curve, the velocity shows no dramatic change at gelation point. The general profile is like that of pure water shown by the dashed line in Fig. 4. The ultrasonic velocity in water, which is the major composition of the sample, had a maximum value around 75℃[9], and this peak was shifted down by the effect of protein molecules as a solute. The temperature at which the ultrasonic velocity reaches its maximum in the gel state was 66℃. The network or the sponge seems to have a negligible contribution to the velocity while it plays a major role in the absorption. Nevertheless, the gelation decreases the velocity. The

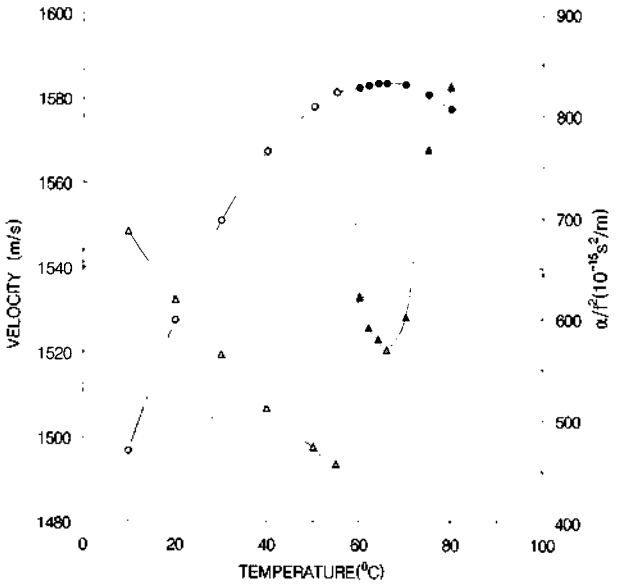

Figure 4. Ultrasonic velocity (○) and absorption coefficient (△) of the egg white at 3 MHz against temperature. The closed symbols indicate the experimental values in the gel state. The dashed line represents the velocity of the pure water.

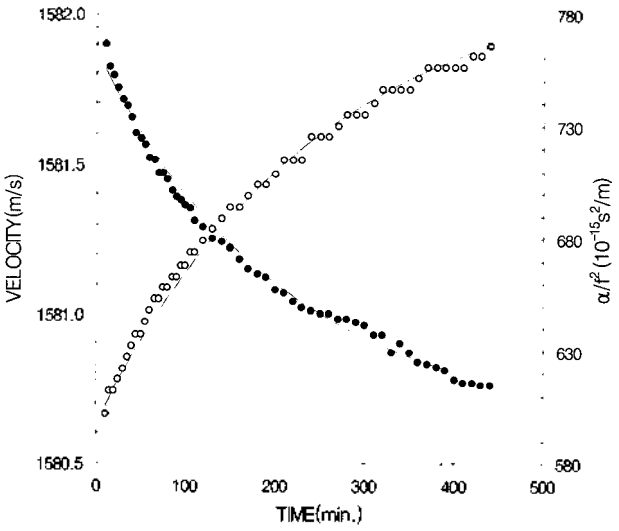

Figure 5. Variation in velocity (●) and absorption (○) in egg white gel observed in aging experiment at 75℃. The solid lines represent the exponential curves fitted to the experiment values.

amount is very small, yet clearly observable in experiment described in the next paragraph.

To study the network-building process in gel, the temperature was quickly raised in the manner of a temperature jump, and the variation in the absorption and the velocity was traced. The closed circles in Fig. 5 show the typical observation of absorption in its gradually increasing region. The network structures may grow up very slowly and the absorption by the network-solution interaction appears gradually. The experiment of Fig. 5 is a real time observation of the network growing process. The solid line is an exponential curve fitted to the experimental points from which the time constant for building the network structure is determined to be 220 (±20) minutes. The time constant obtained at the different temperature agrees well with each other as shown by the open circles in Fig. 6. The total change which is determined from the saturation value of Fig. 5 is a measure of the amount of network newly constructed.

Variation in the velocity was also traced in time as indicated by the open circles in Fig. 5. The pulse-echo overlap technique is sensitive enough to detect the very small decrease less than 1.5 m/s. This decrease gives the contribution of the network to the velocity, which cannot be observed explicitly in the experiment of Fig. 4. When the temperature is suddenly increased, the velocity immediately increases or decreases according to the temperature dependence of the solution, and then the effect of the network begins to decrease the velocity. The mechanism of the decrease in the velocity is not clear at the present time. The decrease in the velocity may be explained as a longitudinal modulus change. The velocity is expressed by $C = (M/\rho)^{1/2}$, where C is the velocity, ρ is the density in the gel state, and M is the longitudinal modulus. The density is not likely to change much with the gel process. The longitudinal modulus is $M = K + 4/3 \times G$, where K is the bulk modulus and G is the shear modulus. When egg white changes from a liquid to a gel state, the constructed network introduces a finite value of the shear modulus, which may increase the longitudinal modulus[10]. Nevertheless, the network structure is rather soft and may decrease the bulk modulus. The overall longitudinal modulus would decrease as the network grows. The time constant for this process was also determined from the velocity curve and shown by the closed circles in Fig. 6, which are in rough agreement with the results obtained from the absorption curve.

C. Hysteresis

The experiments in the previous sections were made with increasing temperature. In Fig. 7, the absorption is

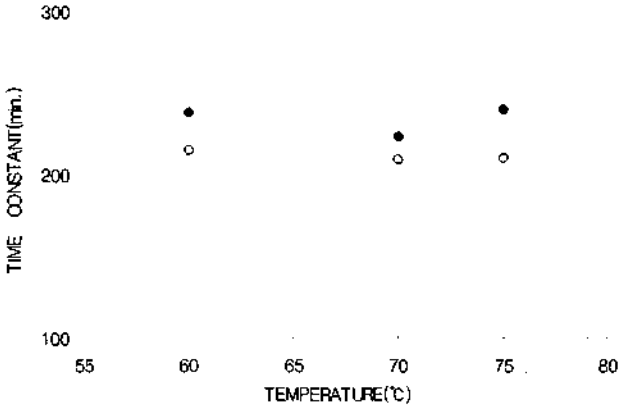

Figure 6. Time constants for ultrasonic velocity (●) and for absorption coefficient (○) at different aging temperatures. The characteristic time for building the network structure is suggested to be 220 (±20) minutes.

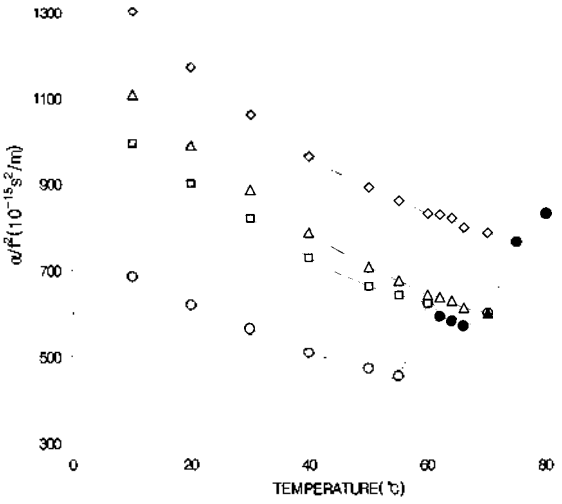

Figure 7. Absorption hysteresis observed in egg white gel. The open circles indicate the experiment values in the sol state, in which the absorption is perfectly reversible. The closed symbols denote the finial values in the aging experiment. The symbols □, △ and ◇ represent the values obtained after aging at 60, 70 and 75℃, respectively.

perfectly reversible in the sol region as shown by open circle. In gel region, however, they present much more complicated feature. The closed symbols denote the final values in the aging experiment. The symbols □, △ and ◇ represent the values obtained after aging at 60, 70 and 75℃, respectively. At temperatures of 62, 64, and 66℃, no gelation process is observed. If we increase temperature up to 60℃ for the first time for instance, and then cool down to 10℃, the absorption varies along the curve A-B in cooling. The path A-B reflects the temperature dependence of the solution in gel and is reversible as long as the temperature is kept below 60℃. If we heat up newly over 60℃, another path is created which is located above the curve A-B as shown Fig. 7.

These hysteresis phenomena can be explained in terms of molecular aggregation in a gel: Above the gelation temperature, the protein molecules in the egg white are denatured and aggregated to make a network structure[5], which causes the change in the absorption and the velocity. If the gel is at a certain temperature T_m for a sufficiently long time, network formation will continue until it arrives at the final equilibrium state at T_m. This process was monitored through the ultrasonic properties, as shown in Fig. 5. The final state of aggregation remains stable as long as the temperature is kept below Tm, and therefore the absorption and the velocity show reversible changes. The first time the gel was heated to temperatures over T_m, aggregation still proceeded and a finer network was formed. The network in the gel became "dense", and the ultrasonic properties had different values. Thus, the thermal history strongly affected the values of the absorption and the velocity in the egg white gel.

IV. Conclusions

Ultrasonic velocity and absorption measurements were carried out to investigate egg white using high-Q ultrasonic resonator method in the frequency range of 0.2-10 MHz and a pulse-echo-overlap technique at 3 MHz which was partially modified in our laboratory. Ultrasonic absorption coefficient is found to be proportional to the 1.25 (±0.02)th power of frequency. The gelation temperature of the egg white is 60℃. At temperatures of 62, 64, and 66℃, there are no variation in velocity and absorption in egg white. The increase in the absorption is described by the interaction between the solvent and the network structure made of protein. The decrease in the velocity is explained by a longitudinal modulus decrease. A hysteresis phenomenon was observed at different temperatures from which aging starts. We have demonstrated that the ultrasonic technique is useful for monitoring the gelation process occurring in egg white.

Acknowledgement

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