Acoustic properties of selected bovine tissues in the frequency range 20–200 MHz

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The acoustic properties of freshly excised bovine liver, heart muscle, and fat are characterized in the frequency range 20–200 MHz by the bioultrasonic spectroscopy system using an ultrasonic transmission comparison method. Significant differences are obtained in the attenuation coefficient, velocity, impedance, and density among these tissues. Measurements of aqueous solutions of bovine hemoglobin are also reported in order to compare the contribution of the protein content to the acoustic properties. The differences among the acoustic properties of liver and heart muscle can be described in terms of their protein contents and other molecular constituents. © *1995 Acoustical Society of America.*

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INTRODUCTION

Studies for biological tissue characterization using ultrasound have been performed extensively in the frequency range below 10 MHz, and the results of these measurements have been compiled.[1,2] However, data over a much broader frequency range of measurements are necessary in order to develop a more complete understanding of ultrasonic wave propagation in biological media. The ultrasonic frequency range of measurement should be expanded in both directions, though this study deals with the higher frequency range.

A bioultrasonic spectroscopy system for tissue characterization for the VHF and UHF ranges has recently been developed.[3] The acoustic properties of attenuation coefficient, velocity, impedance, and density can be determined with high accuracy by an ultrasonic transmission comparison method, wherein distilled water is employed as the reference medium. In this paper, the acoustic properties of bovine liver, heart muscle, and fat are reported in the frequency range 20–200 MHz. Acoustic properties of several aqueous solutions of bovine hemoglobin are also measured and compared with those of the bovine tissues in order to investigate the origins of the acoustic properties of these tissues.

I. MEASUREMENT METHOD

The full description of the measurement method and system is provided elsewhere.[3] Briefly, the experimental configuration for acoustic property characterization is shown in Fig. 1, in which a biological tissue specimen is sandwiched between the parallel surfaces of synthetic silica (SiO_2) buffer rods having ZnO piezoelectric film transducers on their opposite ends. An rf burst signal is supplied to the transmitting transducer, which results in ultrasonic plane waves being produced in the buffer rod. The reflection and transmission signal amplitude and phase at the buffer-rod/sample boundaries are observed. The ultrasonic attenuation coefficient, velocity, impedance, and density are obtained by measuring the transducer outputs V_i, as suggested in the ultrasonic transmission line model shown in Fig. 1, where i describes a particular transmitted or reflected signal voltage amplitude observed at the input or output transducer.

The attenuation coefficient of the sample, α_2, is determined from measurement of the transducer output V_3 in the transmission mode as

$$\alpha_2 = \alpha_2' + \frac{1}{l_2} \ln\left(\frac{(1-|V_1/V_0|^2)|V_3'|ATT_3}{(1-|V_1'/V_0|^2)|V_3|ATT_3'} \right), \tag{1}$$

where ATT_3 is the diffraction loss associated with propagation of ultrasonic waves through the media, and the primed quantities refer to the reference medium of water. The approximation that $ATT_3 = ATT_3'$ can be used for tissue specimens because the velocities for tissues are nearly the same as that of water.

The velocity is obtained by measuring the frequency characteristics of the interference output of V_3 and V_4 in the transmission mode by the gated pulse interference method in which the frequency is swept. The frequency characteristic curve has a number of oscillations having the periodicity Δf. The velocity of the specimen, v_2, is obtained from the relation

$$v_2 = 2 l_2 \Delta f, \tag{2}$$

where l_2 is the thickness of a specimen and the separation distance of buffer rods. The distance necessary for the thickness measurement can be seen from measurements using the reference liquid of distilled water, whose velocity is known. The gap between the two buffer rods can be controlled by a fine stepping motor.

The acoustic impedance of the sample, Z_2, is obtained by measuring the transducer outputs of V_0 and V_1 and using the relation

$$Z_2 = Z_1(1-|V_1/V_0|)/(1+|V_1/V_0|), \tag{3}$$

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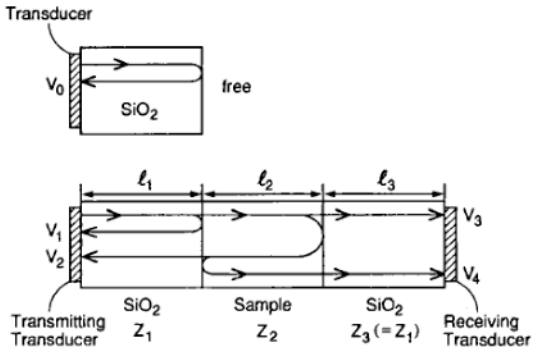

FIG. 1. Definition of transducer outputs V_i for measuring acoustic properties of tissues.

where Z_1 is the known acoustic impedance of the SiO_2 buffer rod.

The density of the sample, ρ_2, is calculated from the measured values of v_2 and Z_2 from the equation

$$\rho_2 = Z_2 / v_2 . \tag{4}$$

All the acoustic parameters, viz., the attenuation coefficient, velocity, impedance, and density, are measured simultaneously by this method. Measurement errors due to diffraction losses in the acoustic media and to mode conversion at the buffer-rod/sample interfaces are corrected by using water as the reference medium. Techniques of precise mechanical alignment of the parallelism of the two rod surfaces, movement to adjust the gap distance, and signal processing are involved in order to obtain high measurement accuracy. These techniques are transferred from the line-focus-beam acoustic microscope technology.[4]

The acoustic parameters are measured by the following procedure for the transmission mode. The gap distance of the two buffer rods is first measured by using water as the reference medium and the transducer outputs V_1' and V_3' for water are measured. After the water is removed, the output V_0 is measured, the gap is opened with a stepping-motor-driven translator, the tissue sample is inserted, the gap is reset to exactly the same length, and the outputs V_1 and V_3 for the sample are measured. In order to assure continuous coupling between a tissue sample and the rods, water is dropped onto the sample surface before resetting the gap length.

The measurement accuracy in the present study is estimated to be better than $\pm 0.1\%$ for velocity and $\pm 1\%$ for attenuation, impedance, and density.[3] In order to achieve the desired accuracy, a pair of ultrasonic transducers with low insertion loss suitable for the measured frequency range of operation and a suitable sample thickness are chosen by taking into account the attenuation in the specimen.

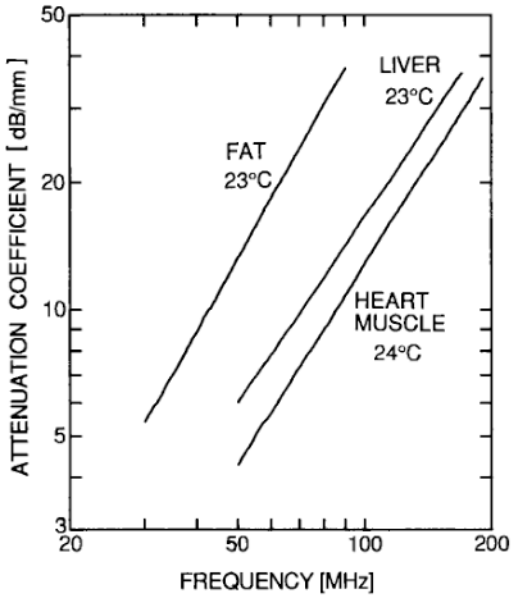

FIG. 2. Typical frequency dependencies of the attenuation coefficients of bovine liver, heart muscle, and fat.

II. EXPERIMENTS AND RESULTS

The measurements were made with samples of freshly excised bovine liver, heart muscle of the left ventricle, and fat, viz., adipose tissue around kidney, within 12 h after slaughter at the abattoir. The operating center frequencies of the ultrasonic transducers are around 150 MHz for measurements of the liver and heart muscle samples, and around 80 MHz for the fat samples. Tissue samples are obtained by cutting perpendicular to the surface of the organs in thicknesses of 1.2–1.4 mm using a razor. The ultrasound propagation direction is perpendicular to the cut surface of the tissue samples.

A number of different samples from each tissue were measured in the temperature range 22–24 °C. Figure 2 shows typical results of the frequency characteristics of attenuation coefficient for bovine liver, heart muscle, and fat samples. It is seen that fat exhibits considerably greater attenuation than do the other two tissues. In the frequency range of measurement, the attenuation coefficients are nearly proportional to the 1.47 power of frequency for liver, the 1.56 power for heart muscle, and the 1.77 power for fat. The results of measurements of the attenuation coefficient, veloc-

TABLE I. Measured attenuation coefficients, velocities, impedances, and densities of bovine liver, heart muscle, and fat at 22–24 °C.

Tissue	α [dB/mm] (70 MHz)	v [m/s] (20–40 MHz)	Z [$\times 10^6$ Ns/m^3] (20–40 MHz)	ρ [kg/m^3]	Number of specimens
Liver	9.73 (9.07–10.2)[a]	1600 (1586–1609)	1.757 (1.718–1.828)	1099 (1083–1138)	10
Heart muscle	8.20 (7.33–8.83)	1566 (1565–1570)	1.591 (1.554–1.628)	1016 (993–1039)	7
Fat	23.4 (22.6–24.1)	1447 (1441–1450)	1.370 (1.332–1.400)	947 (919–967)	5

[a]Example, mean value 9.73 dB/mm, total range of values 9.07–10.2 dB/mm.

ity, acoustic impedance, and density values are summarized for bovine liver, heart muscle, and fat in Table I, where the attenuation coefficients at 70 MHz were used and the velocity and impedance were measured in the frequency range of 20–40 MHz.

The reported values of measured velocities at frequencies below 10 MHz for mammalian liver, heart muscle, and fat are 1573–1614, 1568–1600, and 1410–1510 m/s, respectively,[1,2] which are nearly the same as our measured velocities for each tissue. The data for the attenuation coefficient of bovine liver of 16.7 dB/mm at 100 MHz, in Fig. 2 compare well with the reported value of 13.4 dB/mm at 100 MHz obtained by the SLAM.[5]

In Table I, the range of values obtained for attenuation, acoustic impedance and density, by amplitude measurements, are nearly the same among the three kinds of tissues and somewhat larger than those for velocity, obtained by phase measurements. The velocity for the liver specimens exhibits larger variations than that for the heart muscle and fat specimens. This might be explained by the fact that, although heart muscle exhibits a complex tissue structure associated with its muscle fiber orientations, and fat is relatively homogeneous at all wavelengths considered, liver samples by necessity include vascular structural features which rendered each sample virtually a different specimen.

The values of the exponent on frequency of the attenuation determined for all the samples ranged from 1.45–1.52 for liver, 1.55–1.62 for heart muscle, and 1.73–1.77 for fat, supporting the view that, over a sufficiently broad frequency range, the exponent on frequency may be employed as a parameter for characterizing tissues, and possibly their physiological states.

III. DISCUSSION

To understand the differences in the acoustic properties, obtained among these three kinds of tissues, it is useful to discuss their relationship in terms of their elemental constituents. Table II shows the percentage water content, total protein content, collagen content, and lipid content for liver, heart muscle, and fat tissues.[6,7] The total percentage of water and protein is more than 90% for liver and heart muscle, and about 20% for fat. In order to aid in providing some insight into the potential role of the presence of constituent proteins in the ultrasonic propagation properties of tissues, the acoustic properties of aqueous protein solutions were measured and compared with those of liver and heart muscle. Bovine hemoglobin (Hb) was obtained in powder form (Sigma Chemical Co., H2625) and solutions prepared with singly deionized and distilled water, stirred at room temperature until complete solution was achieved. The solution was placed in the ultrasound spectroscopy chamber and allowed to come to thermal equilibrium before the measurements were made. The concentration of the Hb by weight of the specimen solution was taken as the preparation formulation of the addition of a specified weight of Hb powder to a predetermined volume of water. The operating center frequency of the ultrasonic transducers was around 220 MHz. The entire process required 5 h.

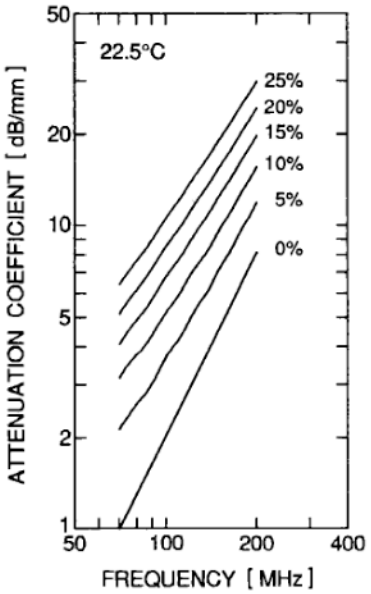

FIG. 3. Frequency dependence of attenuation coefficients versus concentration of aqueous solutions of bovine hemoglobin.

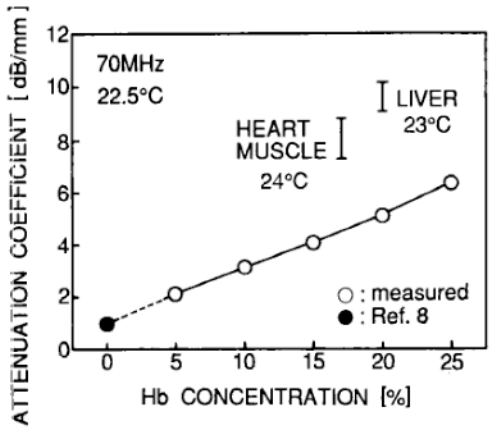

FIG. 4. Attenuation coefficient versus concentration in aqueous solutions of bovine hemoglobin.

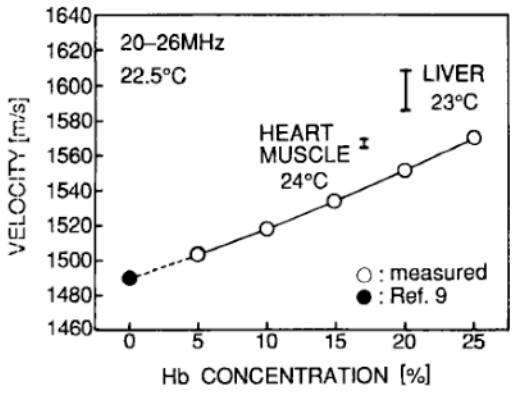

FIG. 5. Velocity versus concentration in aqueous solutions of bovine hemoglobin.

TABLE II. Water, protein, collagen, and lipid contents for mammalian liver, heart muscle, and fat.[6,7]

Tissue	Water (%)	Protein (%)	Collagen (%)	Lipid (%)
Liver	68–78	20–21	0.1–1.3	1.1–11.5
Heart muscle	77–78	17	0.4–1.6	2.7–17
Fat	10–19	5–7	Trace	80

Figure 3 shows the measured frequency characteristics of attenuation coefficient as a function of hemoglobin concentration in the range 5%–25% dry weight. As the concentration increases, the attenuation values increase and the exponent on frequency decreases monotonically, for example, from 1.70 to 1.52 around 150 MHz. The curve for the 0% aqueous solution of hemoglobin is from the published data for water,[8] and the attenuation coefficient is proportional to f^2. It is also seen that the frequency exponent increases with frequency for all hemoglobin concentrations.

The empirical results of the attenuation coefficient, velocity, acoustic impedance, and density for several aqueous solutions of hemoglobin are shown in Figs. 4–7, respectively. The open circles denote the results measured at 22.5 °C and the closed circles are the published data of water.[8–10] The attenuation coefficients at 70 MHz are used for comparison, and measurements of the velocity and impedance were made in the range of 20–26 MHz. The measured results for liver and heart muscle in Table I are also plotted at the corresponding protein concentration of 20% and 17%, respectively (Table II).

It is seen that each of the four parameters increases with the hemoglobin concentration. As the hemoglobin concentration increases 1%, the parameter values between the concentration of 0% and 25% increase approximately 0.22 dB/mm for the attenuation coefficient, 3.2 m/s for the velocity, 7.2×10^3 Ns/m^3 for the impedance, and 2.6 kg/m^3 for the density, respectively. It should be noted that the increase in concentration leads to a nonlinear increase in each of the parameters due, most likely, to intermolecular interactions. Particularly, ultrasonic absorption in aqueous solutions of the protein bovine serum albumin has been shown to be nonlinear from 0% concentration onward.[11]

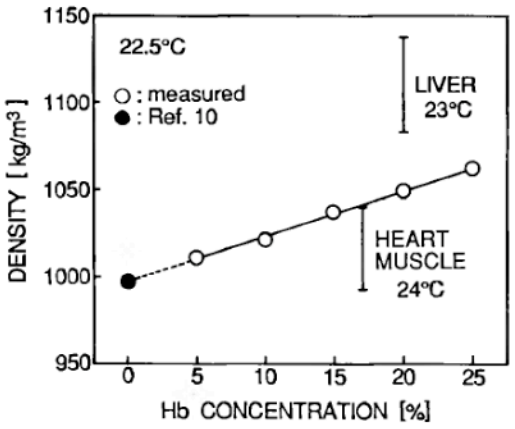

FIG. 7. Density versus concentration in aqueous solutions of bovine hemoglobin.

The differences among the acoustic properties of liver and heart muscle may be considered to be associated with their protein contents, as the four parameters of liver are greater than those of heart muscle, whose protein concentration is the lesser of the two. The values of the exponent on frequency, as a function of frequency, are also well explained with the aqueous solution data, as shown in Fig. 8. Liver, with the greater protein content, has the lesser exponential values for the exponent on frequency than does the heart muscle tissue. These differences may be considered to be due to differences in tissue structure, e.g., scattering from cellular clusters, geometry of collagen deposition, to the presence of different proteins, and to the fact that other components of tissue, such as lipids, may play a significant role in determining the values for these parameters.[7,12]

IV. CONCLUDING REMARKS

The acoustic properties of attenuation, velocity, impedance, and density of bovine liver, heart muscle, and fat have

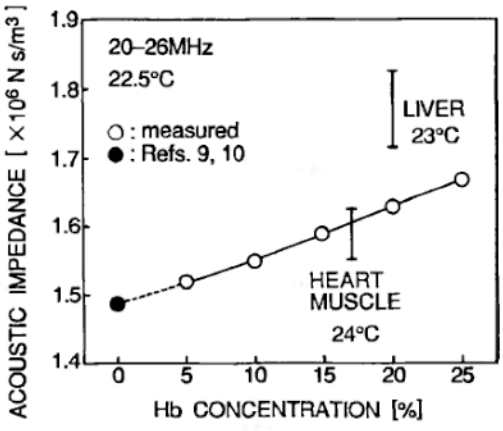

FIG. 6. Acoustic impedance versus concentration in aqueous solutions of bovine hemoglobin.

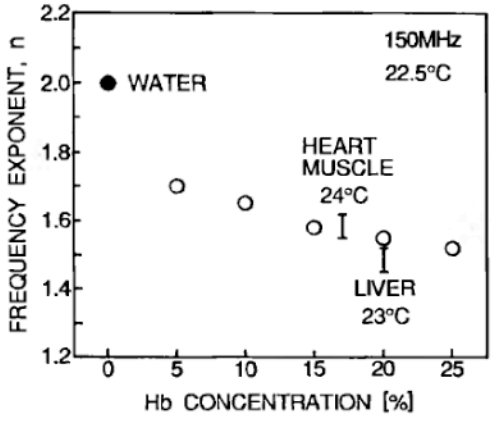

FIG. 8. Exponent n on frequency in attenuation coefficients at 150 MHz versus concentration in aqueous solution of bovine hemoglobin.

been determined in the frequency range 20–200 MHz by the bioultrasonic spectroscopy system employing an acoustic transmission line comparison method. The four parameters of the several aqueous solutions of hemoglobin were also measured in order to aid in understanding the acoustic property values of the tissues in relation to their molecular constituents. All the parameters were observed to increase with the hemoglobin concentration, and all the four parameters of liver tissues are greater than those of heart muscle tissues, whose protein concentration is the lesser of the two. These results support the view that the exponent on frequency may be employed as a characterizing parameter of the attenuation coefficient.

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