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VHF/UHF Range Bioultrasonic Spectroscopy System and Method

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Abstract—**A new system and method for characterizing biological tissues *in vitro* and liquids in the VHF and UHF ranges is described. Bulk acoustic properties such as the sound velocity, attenuation, acoustic impedance, and density are determined in reflection and transmission modes, with the biological tissue/liquid specimen sandwiched between the parallel surfaces of synthetic silica glass buffer rods having ZnO piezoelectric film transducers on their opposite ends. The method is an ultrasonic transmission line comparison method wherein the reference medium is distilled water, for which all acoustic properties are known. Measurement errors due to diffraction losses in the acoustic media and to mode conversion at the buffer/sample interfaces are corrected. Special techniques for achieving precise parallelism between the two rod surfaces, for movement to adjust the gap distance, and for signal processing are employed in order to obtain high measurement accuracy. Attenuation and reflection coefficients are determined using the gated pulse echo method. The sound velocity is determined with the gated pulse interference method by sweeping the ultrasonic frequency, or by changing the gap distance. Results of measurements on castor oil, cottonseed oil, silicone oil, and bovine liver, in the frequency range from 10 to 500 MHz, are presented and compared with results of earlier reports.**

I. Introduction

ULTRASONIC tissue characterization studies have been conducted extensively, particularly in association with its contribution to medical diagnostic and therapeutic applications. Ultrasonic tissue characterization generally means detailed knowledge of the acoustic properties of velocity and attenuation, including absorption and scattering, as functions of frequency and the state variables. These properties are related to the viscoelastic mechanisms, the structural features, and molecular constituents [1]. Tissue characterization studies have been performed mainly at frequencies lower than 10 MHz, though some measurements have been made at 100 MHz; the methods and systems have been summarized [2]. The accumulated results have been compiled [3], [4] and have made significant contributions to bioultrasonics. Further studies are needed to focus on the interaction between biological tissues and ultrasound at even high frequencies to enable detailed interpretations at the lower frequencies. It is also desirable to develop instrumentation for tissue characterization and imaging with high spatial resolution to characterize tissues in the microscopic region.

Only a few bulk studies of liquid specimens have been reported at the higher frequencies. Dunn *et al.* measured ultrasonic absorption for caster oil, cottonseed oil, and silicone oil with the transient thermoelectric method over the wide frequency range from 4 to 2000 MHz [5]. Javanaud *et al.* measured the absorption coefficient of egg yolk and egg white using the pulse method in the frequency range from 2 to 124 MHz [6], but the measured results lacked sufficient accuracy for mechanism interpretation. Recently, Choi *et al.* [7] measured the ultrasonic absorption of aqueous solutions of bovine serum albumin over the broad frequency range from 0.1 to 1600 MHz using four different methods, viz., the plano-concave resonator, plano-plano resonator, Bragg reflection, and high-resolution Bragg reflection methods. Optical diffraction methods, while suitable for aqueous solutions, are not applicable for optically opaque materials.

An early approach to tissue characterization at higher frequencies involved the ultrasonic absorption microscope employing transient thermoelectricity for detection [8]. With the progress in ultrasonic technology for transmission and reception at very high frequencies, two types of acoustical imaging systems, viz., the scanning laser acoustic microscope (SLAM) [9] using plane waves and the mechanically scanned acoustic microscope (SAM) [10] using focused waves, have become available for application in this area. A SLAM system has been employed for measurement of velocity and attenuation in tissues [11]–[15], but its practical application has been largely confined to a single frequency; 100 MHz. SAM systems have received attention for application because of their higher resolution in imaging. Although investigators have expended much effort in developing methods for measuring acoustic properties and for interpreting the detailed images obtained [16]–[25], SAM technology remains in an early stage for use in quantitative tissue characterization. Appropriate systems and methods need to be developed in response to measurement principles based on models in which the effects of viscoelasticity, relaxation, scattering, and nonlinearity on measurements are taken into consideration, together with related questions and problems, such as sample preparation, thickness measurement, etc. It is felt that basic and reliable data on the bulk properties of tissues, and their pathologies and useful phantoms, will be measured at the frequencies of interest and that SAM systems will emerge as most valuable.

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It is apparent that there exists a need for obtaining basic and reliable data on the acoustic properties in the VHF and UHF ranges, not only for use in bulk studies but also for the development of SAM methodology in tissue characterization at the microscopic level [18], [26]–[28]. The system and method to be developed must be suitable for use with excised biological tissues that may have such characteristic acoustic features as high attenuation and low velocity; may have a broad range of physical properties, such as deformability, and chemical components; and have well defined structures at the tissue, cellular, and molecular levels.

This paper describes the development of such a method of bioultrasonic spectroscopy and system using radio frequency (RF) tone burst pulses. This system and method can determine the frequency characteristics of velocity, attenuation, and acoustic impedance, and measure the density simultaneously with high accuracy. Measurements are demonstrated with caster oil, cottonseed oil, silicone oil, and bovine liver, in the frequency range from 10 to 500 MHz, and the results are compared with those of earlier studies.

II. Method

The method described here involves accurately measuring the acoustic properties of liquids and biological tissue specimens *in vitro*, using distilled water as the reference medium whose acoustic properties, viz., velocity and attenuation coefficient, and state properties are well-known as a function of temperature and frequency [29], [30]. An ultrasonic transmission line, as shown in Fig. 1, is constructed for the measurements in which a sample of biological tissue/liquid is sandwiched between the parallel surfaces of synthetic silica glass (SiO_2) buffer rods having ultrasonic transducers on their opposite ends. The acoustic properties are measured using longitudinal waves of RF pulses. This method has the great advantage that thickness measurement of tissue specimens, required for accurate determination of acoustic properties, can be introduced into the procedure. In addition, measurement errors due to diffraction losses in the acoustic media, mode conversion on transmission and reflection at the buffer/sample interfaces, and misalignment of the two buffer rods can be minimized with the use of distilled water whose velocity is close to those of the biological soft tissue and liquid specimens. All the acoustic parameters of tissues, viz., velocity, attenuation, acoustic impedance, and density, can be determined through a series of measurements.

Description of the measurement method is made with the aid of the ultrasonic transmission line shown schematically in Fig. 1. The first buffer rod with the ultrasonic transducer TR(1), a sample of water or specimen, and the second buffer rod with transducer TR(2) are the media 1, 2, and 3, respectively. Each medium $i(= 1, 2, 3)$ with the acoustic loss has complex characteristic acoustic impedance Z_i, length ℓ_i, and propagation constant $\gamma_i = \alpha_i + j\beta_i$, where α_i and β_i are the attenuation coefficient and phase constant, respectively. The quantity β_i is written as $2\pi f/v_i$ where v_i is the acoustic velocity of medium i. Partial reflections and transmissions occur at the a-a' and b-b' interfaces. The reflection coefficient

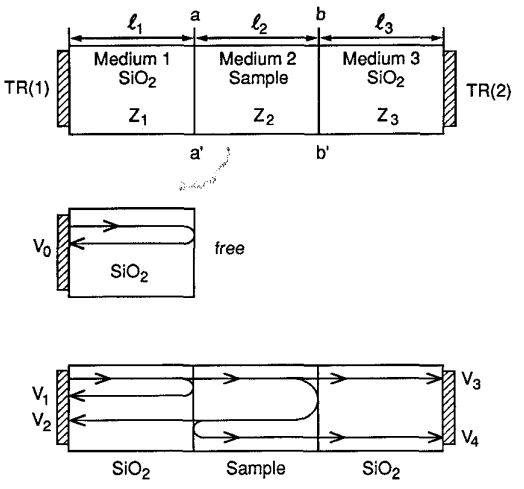

Fig. 1. Ultrasonic transmission line for determining acoustic properties of biological tissues/liquids and definitions of transducer outputs V_i.

R_{ij} and the transmission coefficient T_{ij} at the interface between the media i and j are, respectively, defined as

$$R_{ij} = (Z_j - Z_i)/(Z_j + Z_i), \tag{1}$$

$$T_{ij} = 1 + R_{ij}, \tag{2}$$

when ultrasonic waves are incident from medium i to medium j. The five transducer outputs $V_k(k = 0{\sim}4)$ necessary for measurements, corresponding to the different wave propagation paths and conditions as shown in Fig. 1, can be represented as follows, with diffraction losses of $\mathrm{ATT}_k(k = 0{\sim}4)$ in the corresponding paths. The transducer output V_0 for the free boundary condition at the a-a' interface is

$$V_0 = A{\cdot}R_{\mathrm{free}}\mathrm{ATT}_0\exp(-2\gamma_1\ell_1), \tag{3}$$

where A is the arbitrary transmitted amplitude, $R_{\mathrm{free}} = -1$, and $\mathrm{ATT}_0 = \mathrm{ATT}_1$. When a sample is interposed between the two media, the outputs V_1 and V_2, detected by the TR(1), corresponding to the two signals reflected at the a-a' and b-b' interfaces, respectively, are

$$V_1 = A{\cdot}R_{12}\mathrm{ATT}_1\exp(-2\gamma_1\ell_1), \tag{4}$$

$$V_2 = A{\cdot}T_{12}R_{23}T_{21}\mathrm{ATT}_2\exp(-2\gamma_1\ell_1 - 2\gamma_2\ell_2). \tag{5}$$

The outputs V_3 and V_4 detected by the TR(2), corresponding to the two signals transmitted at the a-a' and b-b' interfaces as seen in Fig. 1 are, respectively,

$$V_3 = B{\cdot}T_{12}T_{23}\mathrm{ATT}_3\exp(-\gamma_1\ell_1 - \gamma_2\ell_2 - \gamma_3\ell_3), \tag{6}$$

$$V_4 = B{\cdot}T_{12}R_{23}R_{21}T_{23}\mathrm{ATT}_4\exp(-\gamma_1\ell_1 - 3\gamma_2\ell_2 - \gamma_3\ell_3), \tag{7}$$

where B is the arbitrary received amplitude.

SiO_2 is a suitable material for the buffer rods (media 1 and 3) in the transmission line, as it has the following advantageous acoustic properties: 1) well-known acoustic properties, 2) relatively low attenuation at higher frequencies, and 3) appropriate acoustic impedance for relatively efficient acoustic transmission at the interface between the buffer rod and the sample of water or tissue.

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A. Sound Velocity

The velocity of sound of the tissue/liquid sample, v_2, with the thickness ℓ_2, can be measured with the double-pulse interference method [31] using the outputs V_1 and V_2 in the reflection mode or using the outputs V_3 and V_4 in the transmission mode. To obtain an interference, a second RF pulse signal is transmitted but delayed in time with respect to the first RF pulse signal until the two output pulses overlap (see Fig. 6). The interference output is measured by sweeping the ultrasonic frequency. A series of interference maxima and minima are observed in the frequency domain for the fixed gap distance between the two buffer rods or the thickness of tissue/liquid specimen. The velocity is determined with the interference periodicity in frequency Δf by

$$v_2 = 2\ell_2 \Delta f. \tag{8}$$

This method is called here the f-interference method. It should, however, be noted that in this method only the average value of velocity can be measured for the swept frequency range in the case of a dispersive specimen. Sometimes, measurement of velocity dispersion characteristics is important for analyzing acoustic properties of tissues or biological aqueous solutions.

A second interference method [32], viz., the z-interference method, for measuring the specimen acoustic velocity at a single frequency can also be carried out. The interference output results from addition between the transducer output V_2 in the reflection mode or V_3 in the transmission mode, as a function of the gap distance z, and the electrical reference signal divided from the signal generator (see Fig. 8). Similar interference maxima and minima as in the f-interference method are observed in the distance domain. The velocity is determined with the interference periodicity in distance Δz in the transmission mode by

$$v_2 = f \cdot \Delta z. \tag{9}$$

B. Attenuation Coefficient

Attenuation can be determined as a function of frequency by the pulse echo method, using the output V_2 in the reflection mode and the output V_3 in the transmission mode.

Using (1) and (2), the equations for the outputs V_2 and V_3 can be rewritten as

$$V_2 = -A \cdot R_{12}(1 - R_{12}^2)\mathrm{ATT}_2 \exp\{-2(\gamma_1\ell_1 + \gamma_2\ell_2)\}, \tag{10}$$

$$V_3 = B(1 - R_{12}^2)\mathrm{ATT}_3 \exp\{-(\gamma_1\ell_1 + \gamma_2\ell_2 + \gamma_3\ell_3)\}. \tag{11}$$

The reflection coefficient R_{12} at the SiO_2/sample interface can be determined from the measurements of V_0 and V_1 as

$$V_1/V_0 = R_{12}/R_{\mathrm{free}} = -R_{12}. \tag{12}$$

The ratio of V_2 for the tissue/liquid sample to V_2' for the reference medium of water can be taken to obtain

$$V_2/V_2' = \frac{R_{12}(1 - R_{12}^2)\mathrm{ATT}_2}{R_{12}'(1 - R_{12}'^2)\mathrm{ATT}_2'} \cdot \exp(-2\gamma_2\ell_2 + 2\gamma_2'\ell_2'), \tag{13}$$

where all the primed parameters identify quantities for water. The velocities of biological soft tissues and liquids are nearly equal to that of water, so that the approximation of $\mathrm{ATT}_2 = \mathrm{ATT}_2'$ may be introduced into (13). If measurements can be carried out under the condition of $\ell_2 = \ell_2'$, the attenuation coefficient α_2 for a tissue or liquid sample in the reflection mode can be expressed finally by

$$\alpha_2 = \alpha_2' + \frac{1}{2\ell_2} \cdot \ln\left\{\frac{(1 - |V_1/V_0|^2)|V_1||V_2'|}{(1 - |V_1'/V_0|^2)|V_1'||V_2|}\right\}. \tag{14}$$

The attenuation coefficient α_2 can thus be determined by making a series measurements of the outputs V_0, V_1, and V_2 for a sample of tissue and for the reference medium distilled water. It is readily seen from the above-described comparison method that possible measurement errors such as diffraction losses in the buffer rods and sample, energy loss due to mode conversion at the interfaces, and additional loss due to misalignment of the mechanical system can be corrected and/or minimized.

In a similar manner, the equation for α_2 in the transmission mode is obtained

$$\alpha_2 = \alpha_2' + \frac{1}{\ell_2} \cdot \ln\left\{\frac{(1 - |V_1/V_0|^2)|V_3'|}{(1 - |V_1'/V_0|^2)|V_3|}\right\}. \tag{15}$$

For practical considerations, the transmission mode is usually more useful for high attenuation tissues and/or at higher frequencies because of lesser total attenuation.

C. Acoustic Impedance and Density

As described above, the reflection coefficient R_{12} can be measured. With the known acoustic impedance of SiO_2, Z_1, the acoustic impedance of the unknown, Z_2, can be calculated by

$$Z_2 = Z_1(1 + R_{12})/(1 - R_{12}), \tag{16}$$

and the density ρ_2 can be determined with the velocity v_2 as

$$\rho_2 = Z_2/v_2. \tag{17}$$

III. System and Measurement Procedure

A. System

An advanced system, satisfying the requirements for accurate measurements at higher frequencies, can be realized by utilizing the pulse mode measurement techniques previously developed [33], together with all the techniques developed for quantitative characterization of solid materials by line-focus-beam (LFB) acoustic microscopy [34], such as precise mechanical alignment and movement and signal processing. A block diagram of the system is shown in Fig. 2. The system is broadly divided into the plane-wave ultrasonic devices, the pulse mode measurement system for transmitting and receiving electrical signals, the mechanical system with controlling circuits to obtain sufficient parallelism between the two buffer rod surfaces and precise translation, the temperature measurement system, and the computer to control the whole system and process the recorded data.

Several pairs of plane-wave ultrasonic devices, with broadband characteristics in pulse mode operation, were used to

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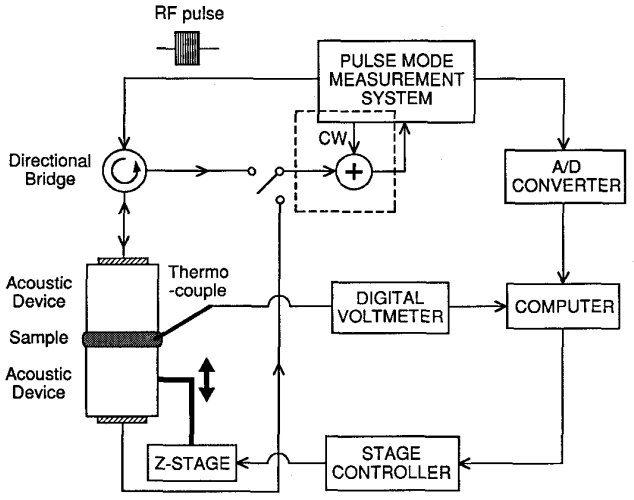

Fig. 2. Block diagram of the bioultrasonic spectroscopy system.

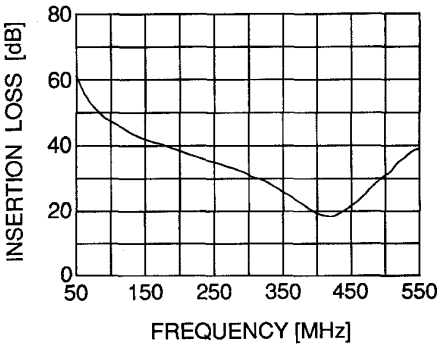

Fig. 3. Typical frequency characteristics of insertion loss of a plane wave ultrasonic device consisting of a ZnO film transducer fabricated on an SiO_2 buffer rod. ZnO film thickness = 6.3 μm, transducer diameter = 1.5 mm.

cover the frequency range from 10 to 1000 MHz. The devices consist of ZnO piezoelectric film transducers fabricated by DC sputtering on one end surface of each of the SiO_2 buffer rods. The buffer rods are 8 mm long and have a sufficiently larger diameter than 8 mm to avoid influence from undesired spurious signals resulting from internal reflections and diffraction. Typical frequency characteristics of insertion loss of the device with a ZnO film thickness of 6.3 μm and a transducer diameter of 1.5 mm are shown in Fig. 3.

The pulse mode measurement system is described in detail elsewhere [33]. In order to realize greater stability in the measurements, the new system constructed for this research investigation employs a spectrum analyzer (Model HP-8568B, Hewlett-Packard Co.) as the receiver and a tracking generator (Model HP-8444A, Hewlett-Packard Co.) as the CW signal generator, operating in the frequency range 500 kHz to 1.5 GHz. Two types of RF switches for the RF pulse modulator and the RF gate are used to obtain a sufficient on-off ratio of more than 100 dB, viz., Schottky barrier diode switches (Model S1, Watkins-Johnson Co.) at the lower frequencies from 10 to 500 MHz and PIN diode switches (Model HP-33144A, Hewlett-Packard Co.) at the higher frequencies over 300 MHz. High pass filters with 10-MHz cutoff after the RF switches are always introduced to suppress the leakage components of switching transients. The system can be switched either in the reflection mode or in the transmission mode.

The RF pulse modulator converts the CW signal of the tracking generator, tuned to the spectrum analyzer, into a pulsed RF signal. The RF pulse is applied to the transmitting transducer through the RF SWR bridge (Model 60N50, Wiltron Co.) operating from 5 MHz to 2 GHz. The pulse duration of 0.1–1.2 μs and the repetition frequency of 2 kHz are selected, taking into consideration the employed ultrasonic frequencies and reverberations in the buffer rods. Pulsed ultrasonic plane waves are emitted into the SiO_2 buffer rod. Partial reflections and transmissions occur at each interface. A train of pulse echoes is detected either in the reflection mode or in the transmission mode. The desired RF pulse signal is extracted by the RF gate and fed into the spectrum analyzer with a maximum IF bandwidth of 3 MHz, which detects the peak power of the RF pulse signal. Because of the limited IF bandwidth, the pulse desensitization must be considered for RF pulses with a narrow pulse width [33]. For velocity measurements by the z-interference method, the Power Combiner (Model H-8-4 (2–2000 MHz), ANZAC) is used to produce interference of the RF pulse signal with the continuous-wave electrical reference signal derived from the tracking generator. The RF gate automatically follows the interference output with a delay time corresponding to the wave propagation time in the sample to be measured. The pulsed video output of the spectrum analyzer is converted into a conventional video output using a peak-holding circuit and recorded in the computer through the 12-bit A/D converter.

The mechanical system plays a most important role in realizing accurate measurements at higher frequencies. A very precise mechanical system was constructed for this purpose, composed of two sets of goniometers to provide perfect parallelism between the two surfaces of the buffer rods, a positioning stage with a stepping motor of resolution of 0.025 μm to set the desired distance between the two rods, and two axis translation stages with a stepping motor of resolution of 0.05 μm to adjust the ultrasonic beam axis between the two ultrasonic plane wave devices.

Temperature of biological tissue/liquid specimens and the distilled water reference medium are monitored by a copper-constantan thermocouple during the measurement procedure. The electrical outputs of the thermocouple are measured with the digital voltmeter and transferred to the computer and converted into temperature values.

Each part of the complete system is controlled by the computer with software for measurements. The operating frequency of the spectrum analyzer and the tracking generator is set by the computer. In order to increase the precision of the frequency in measurements of velocity and the frequency dependence of attenuation, an experimental procedure was adopted under which measurements are made repeatedly by changing the set-up frequency for the desired range. The necessary data on velocities and attenuation coefficients of distilled water, as a function of temperature, are stored in the computer. All the acoustic parameters are determined from a series of experiments according to the measurement method.

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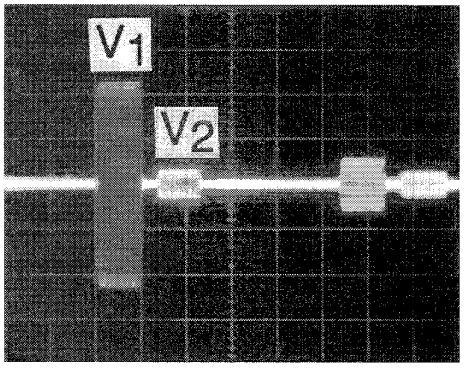
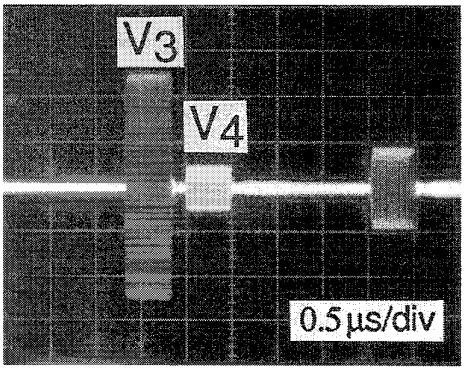


(a)



(b)

Fig. 4. RF pulse echoes observed for distilled water with a gap distance of 500 μm in reflection mode (a) and transmission mode (b).

B. Measurement Procedure

Firstly, careful alignment of the parallelism of the two surfaces of the buffer rods is made in the reflection mode and then the ultrasonic beam axis is adjusted in the transmission mode. Oscilloscope traces of waveforms of RF pulses for distilled water, inserted in a gap 500 μm in length, after the mechanical alignments are shown in Fig. 4, as observed (Fig. 4(a)) for the reflection mode and (Fig. 4(b)) for the transmission mode. An RF pulse with a carrier frequency of 220 MHz, modulated by a DC pulse having a width of 0.5 μs and a repetition frequency of 2 kHz, was employed to excite the ultrasonic transducer. The oscilloscope was synchronized with trigger pulses at the repetition frequency. In the reflection mode, the outputs V_1 and V_2 necessary for measurements, are seen in addition to spurious signals of pulse echoes reflected in the first SiO_2 buffer rod (which have 2.7-μs intervals) and in the water (0.67-μs intervals). On the other hand, in the transmission mode, only a train of transmitted pulse echoes including the outputs V_3 and V_4 can be observed. The measurement procedures are similar for both modes. The procedure for the transmission mode is described in detail as it is more suitable for tissues having high attenuation.

The standard procedure for the transmission mode is illustrated in Fig. 5. The gap distance is selected by performing velocity measurements with the f-interference method, as described above. As shown in Fig. 6, the interference between

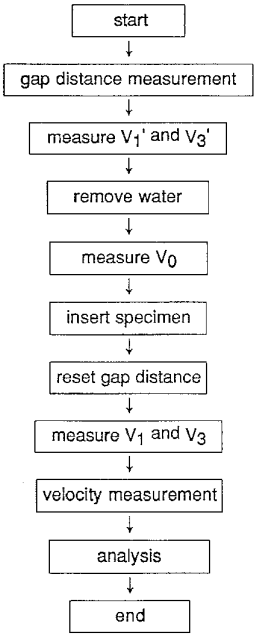


Fig. 5. The measurement procedure for the transmission mode.

the two pulses of V_3 and V_4 is made to occur by the double pulse method and then the interference output is gated out in Fig. 6(d). The frequency characteristics, having the series of interference maxima and minima in Fig. 7(a), are recorded. The gap length can then be measured using the frequency interval Δf obtained from the FFT analysis according to the similar signal processing procedure described previously [34], as shown in Fig. 7(b). A suitable gap distance for the following measurements, equal to the thickness of the biological tissue specimen, is selected. The frequency characteristics of the output V_3', extracted by the RF gate, are determined. By switching the system to the reflection mode, the output V_1' is measured in a similar way. The output V_0 is measured on removal of the water. Then, by opening the gap with the stepping-motor-driven translator, inserting a biological tissue/liquid sample, and resetting the gap to the same length exactly, the output V_1 is measured. These measurements of the three quantities V_1', V_0, and V_1 are used to determine the reflection coefficients R_{12} and R_{12}' which are needed to obtain the final frequency dependence of the attenuation coefficient and the acoustic impedance Z_2 and density ρ_2. By switching the system into the transmission mode, the frequency characteristics of the output V_3 for the specimen is recorded, and the velocity measurement is carried out in the same way as was done for water. Using these measured quantities, all the acoustic parameters can be determined according to the measurement method described in the previous section.

More exact information on characteristics of velocity dispersion for liquids and aqueous solutions can be obtained by using the z-interference method for individually selected frequencies. Fig. 8 shows typical oscilloscope traces of waveforms of signals concerned with the interference output for measurements at 100 MHz. Fig. 9 shows a record of the inter-

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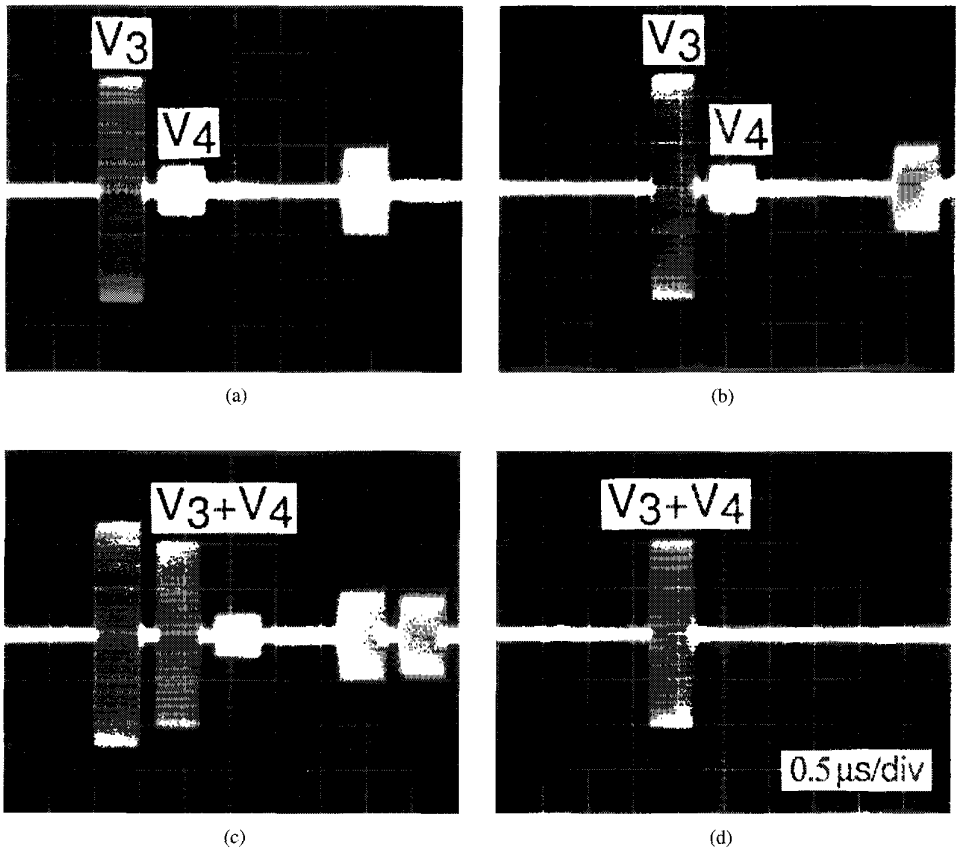

Fig. 6. Interference output by the double-pulse f-interference method (transmission mode): (a) RF pulse echoes by the first RF pulse signal, (b) RF pulse echoes by the second RF pulse signal, (c) superposition of (a) and (b), (d) interference output gated out.

ference output, as a function of distance, with the periodicity that $\Delta z = 15.013$ μm.

C. Distance Calibration

For accurate measurements of velocity and attenuation coefficient, it is necessary to know the length ℓ_2, i.e., the relative position between the two buffer rods. Fortunately, a similar experiment using distilled water, whose velocity is well known as a function of temperature, can be performed to determine the length ℓ_2 in (8), prior to velocity measurements for tissues/liquids with the same thickness as the length for water. Further, we can correct movement errors in the z stage used in the system. If the movement range of the z stage used in measurements at an identical frequency is the same, then the following relation can be made, assuming that the ratio between the measured interference periodicity $\Delta z'$ and the true interference periodicity Δz is the same for water and the sample:

$$\frac{\Delta z_w'}{\Delta z_w} = \frac{\Delta z_s'}{\Delta z_s}. \tag{18}$$

Here, the subscripts w and s stand for water and sample, respectively. If Δz_w is calculated according to (9) using the sound velocity value for water obtained from the literature [29], then Δz_s can be obtained from (18). When this Δz_s is substituted into (9), the corrected velocity value v_2 can be determined.

D. Measurement Accuracy

Measurement accuracy in the system is finally limited by the signal-to-noise ratio of the electrical signals corresponding to the transducer outputs V_i. It is of fundamental importance to choose a pair of ultrasonic transducers with low insertion loss suitable for the measurement frequency range of operation and a suitable gap distance between the two transducers by taking into account the attenuation in the specimen to be measured. In the present study, measurements were conducted with sufficient care to achieve measurement errors within better than $\pm 1\%$ for attenuation, impedance, and density by amplitude measurements and within better than $\pm 0.01\%$ for velocity by phase measurements, as discussed in the next section.

IV. Experiments

A. System Reliability

The velocity and attenuation coefficients of the reference medium water are measured first. The velocity can be determined by (9) using the z-interference method. The velocities

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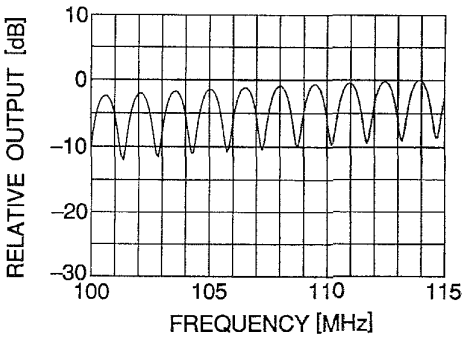
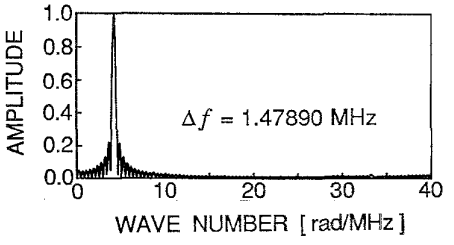


(a)



(b)

Fig. 7. Velocity measurement by the f-interference method (transmission mode): (a) interference output as a function of frequency, (b) spectral distribution to measure frequency periodicity.

were measured 10 times in succession using the ultrasonic frequency of 100 MHz at the temperature of 22.9°C. The gap distance between the two buffer rods was translated from 700 μm to 1000 μm using the mechanical translation stage. The average velocity is 1492.6 m/s, 0.111%, with a maximum deviation of 0.01%, greater than the value of 1490.951 m/s at 4 MHz reported by Kroebel and Mahrt [29]. This difference is believed to be due mainly to displacement errors of the mechanical stage, because water exhibits no velocity dispersion up to 5.5 GHz [35], [36]. In this comparison method, using the reference liquid water, velocity measurements are conducted on both water and the tissue/liquid sample. These kinds of errors can be corrected experimentally. The velocity measurement errors occurring in the z-interference method were estimated to be around $\pm 0.01\%$. Very nearly the same accuracy in velocity measurements can be achieved by the f-interference method because mechanical translation is required in the experimental procedure in this comparison method.

The attenuation coefficient of distilled water is measured directly in the reflection mode. In this case, the attenuation coefficient can be obtained approximately through the following equation, using only the measured values with the sample:

$$\alpha_2 = \frac{1}{2\ell_2} \ln \left\{ \frac{|V_1| \cdot (1 - |V_1/V_0|^2)}{|V_2|} \cdot \frac{\mathrm{ATT}_2}{\mathrm{ATT}_1} \right\}. \qquad (19)$$

The attenuation coefficient α_2 is obtained with an assumption that $\mathrm{ATT}_2/\mathrm{ATT}_1$ is very nearly equal to unity. Accordingly, α_2 must include the measurement error due to diffraction loss. Fig. 10 shows the results for the attenuation of water as a function of frequency at 23°C, using plane wave ultrasonic transducers with an operating frequency around 400 MHz. The attenuation coefficient varies as the square of the frequency in the frequency range from 70 to 500 MHz. The value of α/f^2 is 22.6×10^{-17} s^2/cm at 23°C. This value is approximately 3% less than 23.2×10^{-17} s^2/cm at 23°C calculated from the measured data in the frequency range 7.5 to 67.5 MHz by Pinkerton [30]. Choi and Takagi reported the value of 24.0×10^{-17} s^2/cm at 20°C for the frequency range 100 MHz to 1.5 GHz [35].

The measured results of velocity and attenuation coefficient obtained here are in good agreement with those previously reported. The values for the velocity of water measured precisely by Kroebel and Mahrt [29], and those for the attenuation coefficient reported by Pinkerton [30] are employed as the reference data for measurements using the comparison method.

Further detailed investigations of the attenuation coefficient of distilled water as a function of temperature should be conducted as the acoustic properties are employed as the reference medium in the present system, as well as in the line-focus-beam acoustic microscopy system for quantitative solid materials characterization.

The attenuation coefficient of cottonseed oil was investigated in order to assess the influence of misalignment on measurements. Fig. 11 shows the results when the parallelism of the two rods is altered. The ultrasonic frequency is 100 MHz, the temperature is 23.3°C, and the gap distance between the buffer rods is 300 μm. $\Delta\theta$ in the horizontal axis expresses the relative inclination angle between the two buffer rods, so that when $\Delta\theta = 0$ the two rods are in perfect parallelism. Measurements are made in the reflection mode, and the circles represent the attenuation coefficient values as determined with (14), using water as the reference medium. The squares represent the attenuation coefficient values as obtained with (19) without using the reference medium. When no reference medium is used, $|V_2|$ decreases as $|\Delta\theta|$ increases, so that the attenuation coefficient values shown by the squares appear to increase as $|\Delta\theta|$ increases. On the other hand, in the case where the reference medium is used, even when $|\Delta\theta|$ increases, so long as $|V_2/V_2'|$ is taken to be equal to unity, the attenuation coefficient values shown by the open circles remain very nearly unchanged. The figure shows that the attenuation coefficient values determined using the measured values with water remain roughly fixed for $|\Delta\theta| < 5$ min. Because the present system can easily be adjusted to $|\Delta\theta| < 0.1$ min, measurement errors due to misalignment will not be significant.

B. Determination of Acoustic Parameters

The acoustic properties of biological soft tissues and liquids are, in general, frequency dependent. Thus, there is a need for measurements of frequency dependence over a broad frequency range and this can be accomplished using multiple ultrasonic transducer pairs, each with a different frequency operating range.

Determination of the four acoustic parameters, viz., attenuation coefficient, velocity, acoustic impedance, and density, are reported for castor oil, cottonseed oil, and Dow-Corning-710 (DC-710) silicone oil. Fig. 12 shows the frequency characteris-

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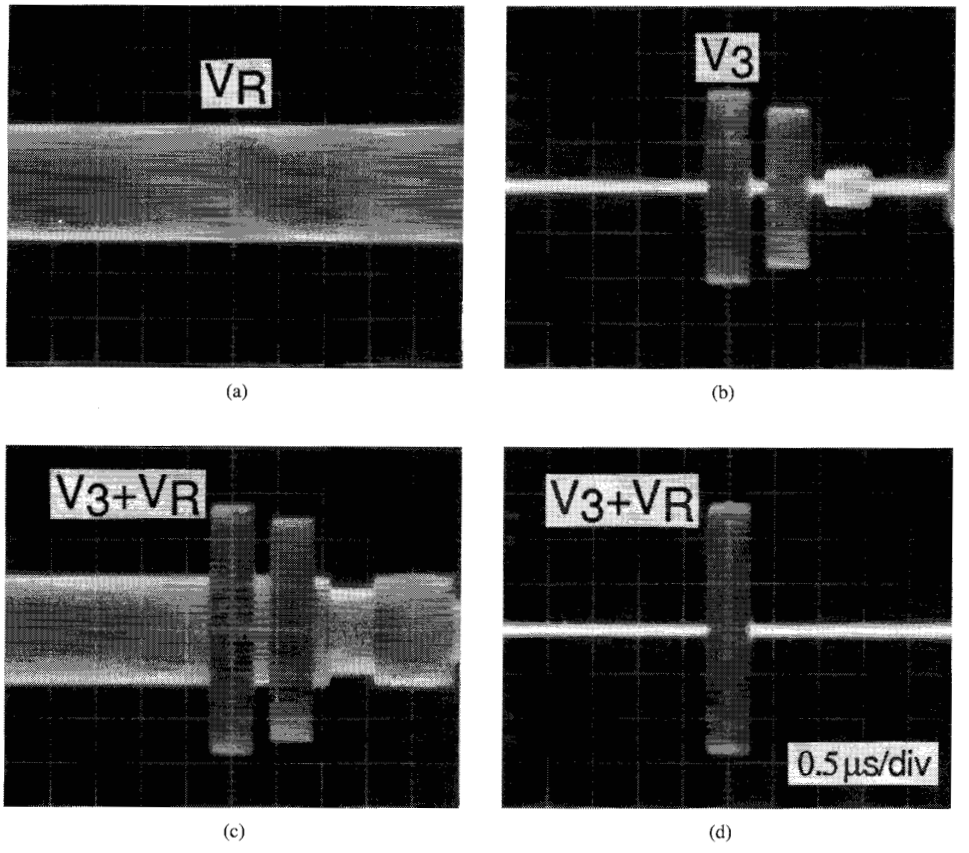

Fig. 8. Interference output by the z-interference method (transmission mode): (a) CW reference signal, (b) RF pulse echoes, (c) superposition of (a) and (b), (d) interference output gated out.

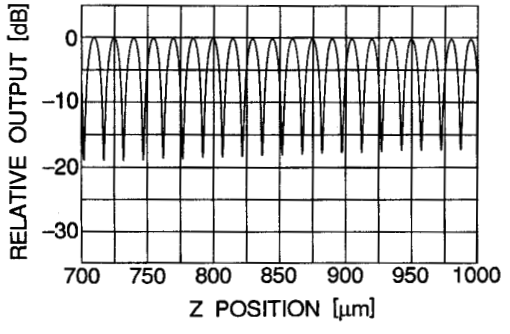

Fig. 9. Interference output as a function of gap distance for velocity measurement by the z-interference method (transmission mode). $f = 100$ MHz.

tics of the attenuation coefficient measured in the transmission mode around 23°C. The system is capable of measuring attenuation coefficient values as high as 600 dB/mm. Experimental data are shown in the ultrasonic frequency range to 250 MHz for castor oil, 400 MHz for cottonseed oil, and 260 MHz for DC-710 silicone oil.

The experimental conditions, for example, for castor oil, were as follows. In the frequency range shown in the figure, transducers with the center frequencies of 80 and 220 MHz were employed. Each pair of the transmitting and receiving transducers operating in the respective frequency ranges was

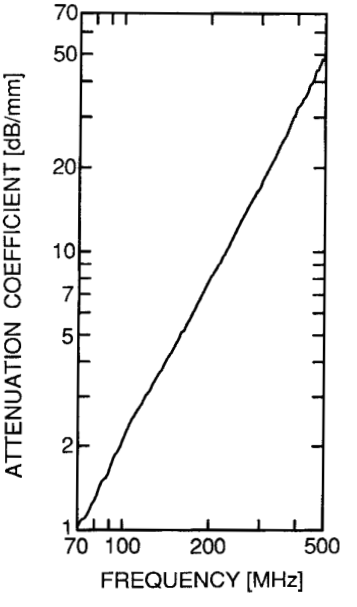

Fig. 10. Frequency dependence of the attenuation coefficient of distilled water at 23°C.

used with the different gap distances, viz., 600 μm for 20–90 MHz and 130 μm for 90–250 MHz. Each oil has a different

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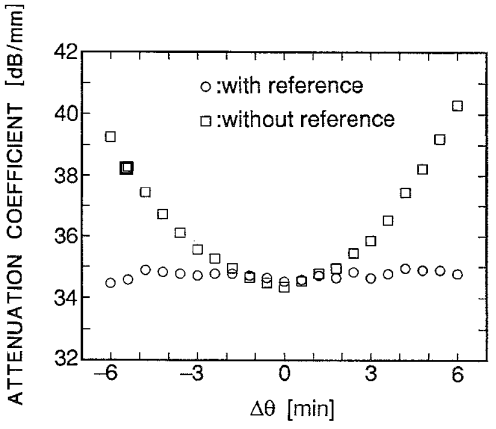

Fig. 11. Influence of misalignment on attenuation measurements of cottonseed oil in reflection mode at 23.3°C. $f = 100$ MHz.

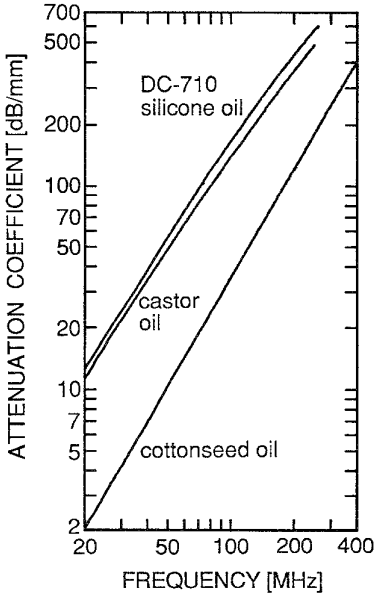

Fig. 12. Frequency dependence of attenuation coefficients of castor oil (23.3°C), cottonseed oil (23.0–23.2°C), and DC-710 silicone oil (22.7–23.0°C).

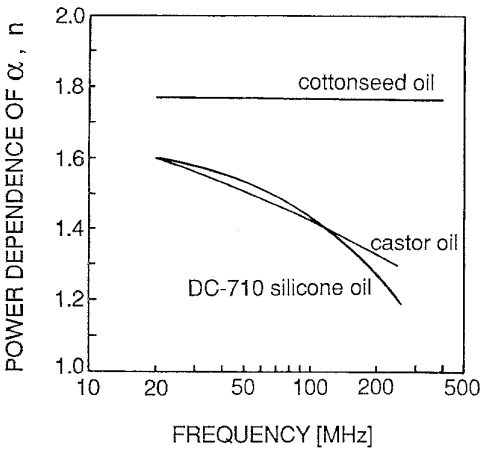

Fig. 13. Frequency dependence of the power on attenuation coefficients for castor oil, cottonseed oil, and DC-710 silicone oil, obtained from Fig. 12.

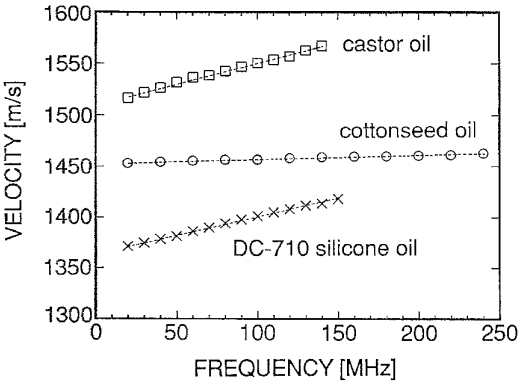

Fig. 14. Velocity dispersion of castor oil, cottonseed oil, and DC-710 silicone oil determined by the z-interference method.

magnitude and frequency dependence of attenuation. Comparing the results among these three oils, DC-710 silicone oil has the greater attenuation. The attenuation values at 100 MHz are 143 dB/mm for castor oil, 35.5 dB/mm for cottonseed oil, and 167 dB/mm for DC-710 silicone oil. They also have significantly different power dependences of frequency, as shown in Fig. 13. The attenuation coefficient for castor oil is roughly proportional to the 1.6 power of frequency at 20 MHz, to the 1.45 power around 100 MHz, and to the 1.3 power at 250 MHz; the attenuation coefficient for cottonseed oil is proportional to the 1.77 power of frequency over the entire frequency range; and the attenuation coefficient for DC-710 silicone oil is proportional to the 1.6 power of frequency at 20 MHz and rapidly dropping to the 1.2 power of frequency at 260 MHz. These results of the frequency characteristics, measured continuously as a function of frequency, exhibit a refinement over the absorption coefficients measured by the transient thermoelectric method [5] a third of a century ago, showing that the frequency dependences of the attenuation coefficients for all these oils are quite different from those of the absorption coefficients. The values reported earlier were: for castor oil—the absorption coefficient was proportional to the 1.66 power of frequency in the frequency range 1 to 500 MHz (30°C), for cottonseed oil—the absorption coefficient was proportional to the square of frequency 3 to 100 MHz (26°C), and for DC-710 silicone oil—the absorption coefficient exhibited a significant change in the power dependence of frequency, measured up to 2 GHz, which appeared to be described by a single relaxation process centered at around 40 MHz (26°C) [5].

Fig. 14 shows the velocities for castor oil, cottonseed oil, and DC-710 silicone oil, measured by the z-interference method. All of these oils exhibit velocity dispersion characteristics. The velocity dispersions values, viz., 0.40 m/s/MHz for castor oil, 0.04 m/s/MHz for cottonseed oil, and 0.36 m/s/MHz for DC-710 silicone oil, were obtained by linear approximation, as shown in Fig. 14. Cottonseed oil has the

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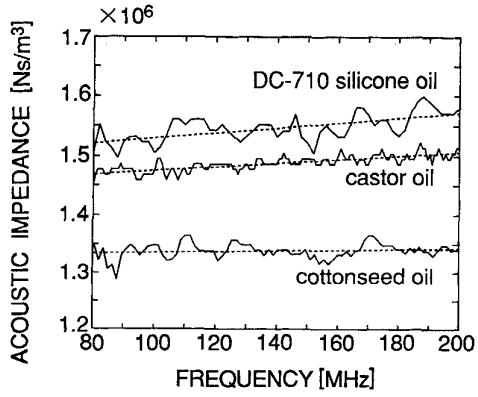

Fig. 15. Frequency dependence of the acoustic impedances of castor oil, cottonseed oil, and DC-710 silicone oil determined from the reflection coefficients. Broken lines: by the method of least squares.

TABLE I
COMPARISON OF DENSITIES MEASURED BY THE ULTRASONIC METHOD WITH THOSE MEASURED BY A PYCNOMETER AT 23°C

Material	Ultrasonic Method	Pycnometer	Difference
Castor Oil	951.3	957.4	-0.64%
Cottonseed Oil	917.4	917.8	-0.04%
DC-710 Silicone Oil	1,092.3	1,100.2	-0.72%

Units in kg/m^3.

least dispersion among these oils, as well as the least attenuation, as shown in Fig. 12. It is well understood from the Voigt model that liquids with greater viscosities should have greater absorption and larger velocity dispersion [20].

Fig. 15 shows the acoustic impedances of these three liquids determined with the known value of SiO_2 acoustic impedance of 13.12×10^6 Ns/m^3 [37] by measuring the reflection coefficient R_{12} in (12). Concerning the velocity dispersion obtained by the phase measurements, as shown in Fig. 14, it is clear that even in amplitude measurements the measured acoustic impedances vary with the frequency. Application of the method of least squares to the measured curves yields the average acoustic impedances with standard deviation σ and the rates of increase for the impedances as: 1.488×10^6 Ns/m^3 with $\sigma = 0.008 \times 10^6$ Ns/m^3 (0.53%) and 320 Ns/m^3/MHz for castor oil; 1.546×10^6 Ns/m^3 with $\sigma = 0.017 \times 10^6$ Ns/m^3 (1.1%) and 419 Ns/m^3/MHz for DC-710 silicone oil; and 1.338×10^6 Ns/m^3 with $\sigma = 0.013 \times 10^6$ Ns/m^3 (0.96%) and 74 Ns/m^3/MHz for cottonseed oil, respectively.

The densities of the oils were obtained by dividing the measured results of acoustic impedances, shown in Fig. 15, by those of velocities, shown in Fig. 14. The average densities are: 951.3 kg/m^3 with a maximum difference of 2.5 kg/m^3 (0.26%) for castor oil, 917.4 kg/m^3 with a maximum difference of 2.5 kg/m^3 (0.27%) for cottonseed oil, and 1,092.3 kg/m^3 with a maximum difference of 0.8 kg/m^3 (0.07%) for DC-710 silicone oil. These values are in excellent agreement with the values measured by a pycnometer as shown in Table I. The measurement accuracy of density by this ultrasonic method can be estimated to be better than 1%.

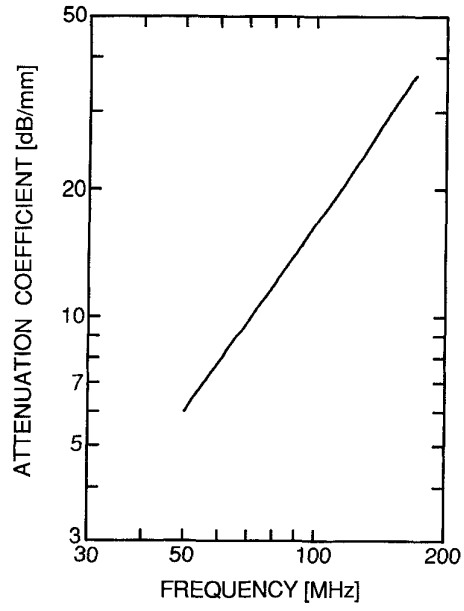

Fig. 16. Frequency dependence of the attenuation coefficient of bovine liver at 23°C.

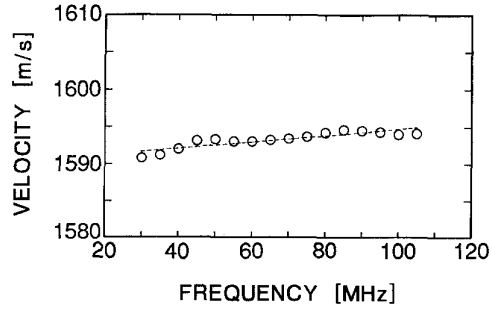

Fig. 17. Velocity dispersion of bovine liver tissue determined by the f-interference method.

In order to demonstrate the usefulness of the method and system for *in vitro* measurements on mammalian tissues and organs, a sample of bovine liver tissue was investigated. The sample was obtained from a commercial slaughterhouse and used within 12 h of slaughter, prepared by cutting the fresh specimen perpendicular to the major surface in a thickness of 1 mm. The operating center frequency of the plane wave ultrasonic transducer used was around 150 MHz, for both the transmitter and the receiver. The sample was measured in the frequency range 50 to 170 MHz at 23°C. Fig. 16 shows the results of determination of the frequency characteristics of the attenuation coefficient. It is seen that the attenuation coefficient increases from being proportional to the 1.41 power of frequency in the frequency range up to about 90 MHz and increases to the 1.52 power at about 170 MHz. This represents the first observation, on a single specimen at a single preparation at a fixed temperature over such a broad frequency range, that the frequency exponent increases with frequency and approaches that for water, the principal constituent of

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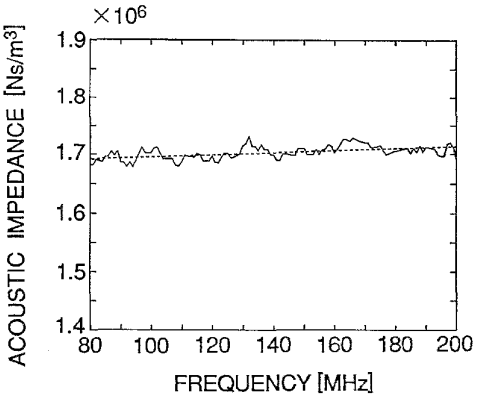

Fig. 18. Frequency dependence of the acoustic impedance of bovine liver tissue determined from the reflection coefficients. Broken lines: by the method of least squares.

liver [38]. The velocity measurements for the tissue, by the f-interference mode, are shown in Fig. 17. The slight velocity dispersion of 0.04 m/s/MHz was observed in this frequency range. The acoustic impedances were determined by amplitude measurements to have an average value of impedance 1.703×10^6 Ns/m^3, with a $\sigma = 0.010 \times 10^6$ Ns/m^3 (0.56%), exhibiting a slight frequency dependence at the increasing rate of 185 Ns/m^3/MHz, as shown in Fig. 18. The average density was measured as 1.063×10^3 kg/m^3 with a maximum difference of 2 kg/m^3 (0.19%). Velocity and attenuation measurements for several kinds of liver, obtained by a SLAM technique to 100 MHz, have been reported. The data reported herein for the attenuation coefficient of bovine liver at 100 MHz, viz., 16.7 dB/mm, compares well with the reported value 13.4 dB/mm [11] following the observation of the increase in the exponent on the frequency dependence. These data also compare well for velocity; 1594.1 m/s at 100 MHz for bovine liver with 1553–1584 m/s reported for rat liver [39].

V. Concluding Remarks

A VHF/UHF ultrasonic spectroscopy system for characterization of excised biological tissues has been developed. The acoustic properties of velocity, attenuation coefficient, acoustic impedance, and density can be determined simultaneously as a function of frequency with high accuracy by an ultrasonic transmission line comparison method, wherein the well-known properties of distilled water are employed as the reference data. The measurement errors such as those caused by misalignment, diffraction loss, and mode conversion at interfaces, can be minimized experimentally. Measurements of the four acoustic parameters have been demonstrated successfully both for samples of castor oil, cottonseed oil, and DC-710 silicone oil, and for a biological sample, viz., bovine liver tissue, in the frequency range 10 to 500 MHz, the highest frequency yet reached for mammalian organs.

It continues to be of fundamental importance to understand the interaction of ultrasonic waves with biological media 1) in terms of the molecular constituents, 2) at structural levels from molecular to organ, and 3) with regard to model aqueous solutions. Accordingly, the system and methodology described herein is expected to make a substantial contribution to future research in the field of biological tissue characterization.

Acknowledgment

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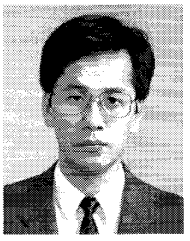
Jun-ichi Kushibiki (M'83), for a photograph and biography, see p. 90 of the January 1995 issue of this TRANSACTIONS.



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