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Ultrasonic spectrometry for the investigation of biological media

R.Kaþys, L.Maþeika, R.Ðliteris, A.Voleiðis

Prof. K.Barðauskas Ultrasound Research Center

Kaunas University of Technology

Introduction

Ultrasound attenuation measurement techniques for biological media are based on the fact that the attenuation coefficient is frequency dependent. This dependence can be expressed as $\alpha(f)=\alpha_0\times f^n$, where f is the frequency, $\alpha(f)$ is the frequency- dependent attenuation coefficient, and α_0, n are the attenuation parameters characterizing the medium. In the case of citrated nonclotted blood n=1.21, α_0=0.15. Other biological liquids exhibit values of n=1.1÷1.3 and α_0=0.01÷0.25 [1]. Analysis of the publications reveals that there is not enough data on the ultrasonic attenuation measurements in biological fluids, and especially on the measurements in pathological fluids. These various fluids exhibit a wide range of physical properties, and it is expected that they would affect a of acoustic properties, which eventually might find use in diagnosis.

In overall investigation of ultrasound attenuation mechanism in biological media the molecular weight, the molecular structure, the biomolecular- solvent and biomolecule - biomolecule interactions [2] have the principal role. In order to characterize completely the properties of biological media, attenuation behavior over a wide range of frequencies must be examined, where relaxation phenomena are observed. To perform accurate measurements of intrinsic attenuation is difficult because, in addition to intrinsic damping, geometric spreading, reflections and scattering may strongly affect propagation of waves. In principle, both the attenuation and velocity are known to be sensitive to changes in material properties, thus measurement of these parameters in a wide range of frequencies is a very effective means of media characterization. In the case of biological media attenuation measurements are more perspective because of small values of ultrasound dispersion.

Experimental approach

For the measurements over a wide range of frequencies it is possible to monitor acoustic behavior of a medium with a monochromatic pulse for a series of discrete frequencies [2,3]. This is a tedious and time consuming procedure.

An alternative modern method employs a broad band acoustic pulse transmission and Fourier analysis techniques to determine media response over a wide frequency range. After propagation through the medium, the acoustic signal will be delayed in the time and attenuated. Due to the dispersion, different frequency components of the pulse will travel at different velocities so distorting the waveform, and as a result of attenuation the strength of the disturbance will depend on a distance.

As measurements are usually performed at distances close to the Fresnel zone, additional distortions of the pulse shape and changes of its frequency content are observed due to the diffraction. The diffraction corrections as well as the measurement procedure will depend on the principle of operation of the experimental cell. The pulse - echo technique and cells with multireflections or buffer rods have been used. An alternative can be the through - transmission method [4,5]. In biological media investigations transducer - reflector distance is small and if the absorption is negligible pulse multireflections are observed. In making accurate attenuation measurements, the misorientation of the transducer with respect to the reflector is of particular importance. Optimal orientation is achieved according to the maximum number of multireflections observed. The first reflection in this series is used as a reference, and others as informative.

The ultrasound attenuation $\alpha(\omega)$ and velocity $c(\omega)$ in biological media are related to each other according to the Kramers- Kronig relationships:

$$\alpha(\omega)=\frac{\pi^2}{2c_0^2}\frac{dc(\omega)}{d\omega}, \qquad (1)$$

$$c(\omega)=c_0+\frac{2c_0^2}{\pi}\int_{\omega_0}^{\omega}\frac{\alpha(\omega)}{\omega^2}d\omega, \qquad (2)$$

where c_0 is the velocity at the frequency ω_0. If the velocity $c(\omega)$ is non - stationary and depends on the measurement moment t_n as in the blood during the clotting process, then the attenuation is also non- stationary, and $\alpha(\omega)\rightarrow\Delta\alpha(\omega, t_n)$.

The ultrasound attenuation coefficient obtained from Fourier analysis is expressed as follows:

$$\alpha(\omega,t_n)=\frac{1}{x_n-x_1}\ln\left|\frac{S_{\text{in}}(\omega,t_n)}{S_{\text{out}}(\omega,t_n)}\right|, \qquad (3)$$

where $S_{\text{in}}(\omega, t_n)$ and $S_{\text{out}}(\omega, t_n)$ are the Fourier transforms:

$$S_{\text{in}}(\omega,t_n)=\int_{t-t_n}^{t}w(t-t_n)u_{\text{in}}(t)e^{-j\omega t}dt, \qquad (4)$$

$$S_{\text{out}}(\omega,t_n)=\int_{t-t_n}^{t}w(t-t_n)u_{\text{out}}(t)e^{-j\omega t}dt, \qquad (5)$$

$w(t)$ is the window function, $u_{in}(t)$ is the reference signal at $x=x_1$, $u_{out(t)}$ is the informative signal at $x=x_n$.

Similarly, from the phase spectra $\Psi(\omega, t_n)$ ultrasound velocity can be obtained:

$$c(\omega,t_n)=\frac{\omega(x_n-x_1)}{\Psi_{out}(\omega,t_n)-\Psi_{in}(\omega,t_n)}. \qquad (6)$$

The experimental setup is shown in Fig.1. The pulser - receiver circuits were designed especially for the excitation and reception of wideband ultrasonic signals. The ultrasonic transducer *Tr* emits a wideband ultrasonic longitudinal pulse which is sent into the biological medium, and after reflection is received by the same transducer. The transmitted signal is amplified and digitized by HP54645A oscilloscope with a sampling rate of 200 MHz. The digitized signal is stored and processed by the Pentium type PC. In the case of investigation of non - stationary media, the experimental data are taken with equal intervals of 1min using the computer clock as a time reference. 64 transmitted signals were digitized and averaged.

Results

Experiments were performed with degassed distilled water and citrated nonclotted blood sample, obtained from the blood bank. Water is a low - absorptive nondispersive standard liquid, and blood is a typical biological medium characterized by an ensemble of relaxation processes.

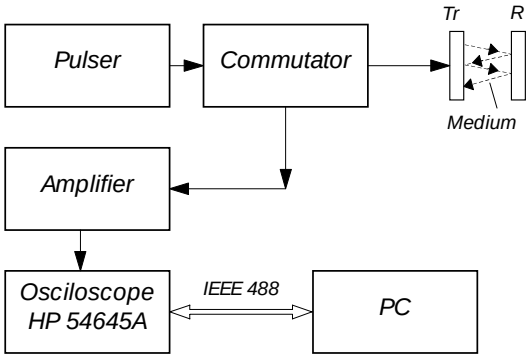

Fig.1. **The schematic of an experimental setup for ultrasonic spectrometry of biological media**

Theoretical dependencies of the ultrasound attenuation on frequency are presented in Fig.2. They were calculated according to the formulae:

$$\alpha_w(f)=23\times10^{-17}\times f^2,\ s^2/cm \text{ (water, 22 °C)},$$

$$\alpha_b(f)=0.15\times f_{MHz}^{1.21},\ dB/cm \text{ (blood, 22 °C)}.$$

The absorption of ultrasound in water is very small as compared with biological media and usually can be measured only at higher frequencies and using large acoustic signal paths.

At the transducer - reflector distance L=15 mm we have observed up to 10 multireflections. The first reflected signal was exploited as the reference signal, and the fifth is one of the possible nine informative signals (Fig.3). A noticeable change in the waveform as a result of interaction with the propagation medium is observed. It is difficult to determine which features of the waveforms

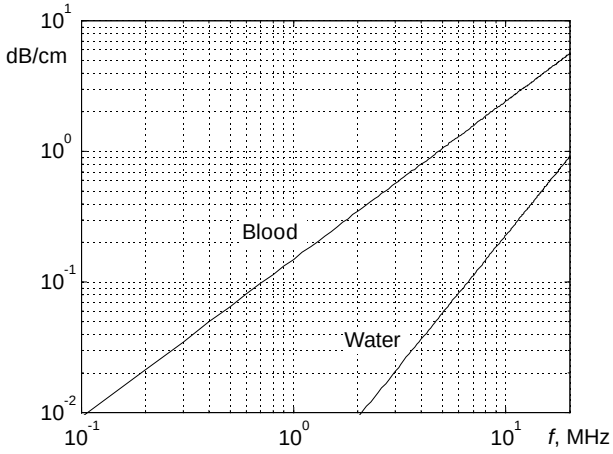

Fig.2**. Theoretical dependencies of the ultrasound attenuation in water and citrated blood**

should be compared for determining the medium attenuation. With the spectroscopic technique, there is no ambiguity as one is always comparing components of the same frequency. As expected (Fig.3), the higher frequency components in the wideband informative pulse tend to be attenuated more than the lower frequency components, and spectrum decreases continuously with increasing frequency.

The average attenuation versus frequency in water obtained from different pairs of spectra is shown in Fig.4, where the reflection coefficient K=0.74 (water - ceramic, water - metal) was taken into account. As the theoretical

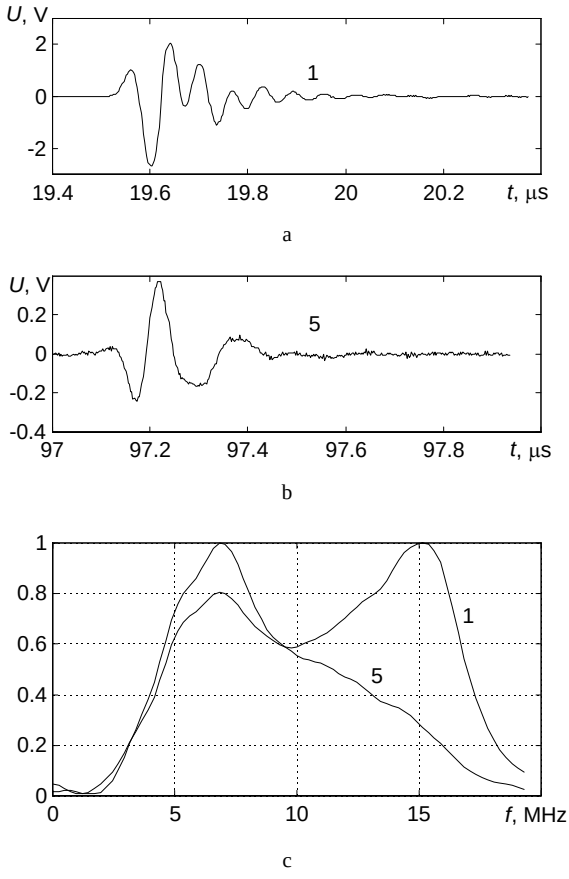

Fig.3. **The first (reference)(a) and fifth (b) wideband pulses in water and their spectra (c)**

dependence of the attenuation in water is well known, it is clear that the results up to 7 MHz are higher due to the diffraction. A digital signal processing facilitates the use of diffraction correction factors for each frequency present in the signal.

Measurements in citrated blood were carried out with a different cell and transducer. As a result of a higher absorption, only three reflections were observed, though the acoustic base was decreased to L=4.5mm. The reference and the second informative pulses are shown in Fig.5. As seen, the informative pulse has lost its high frequency components.

Attenuation in blood as a function of frequency is shown in Fig. 6. Here the diffraction also tends to increase results in low frequency region below 5 MHz. The results

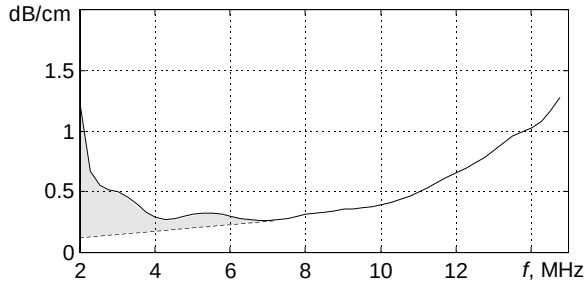

Fig.4. **Average ultrasound attenuation versus frequency in water**

over 17 MHz are unstable due to the low values of the signal/noise ratio.

Conclusions

The digital ultrasonic spectroscopic technique developed enables to perform measurements of ultrasound

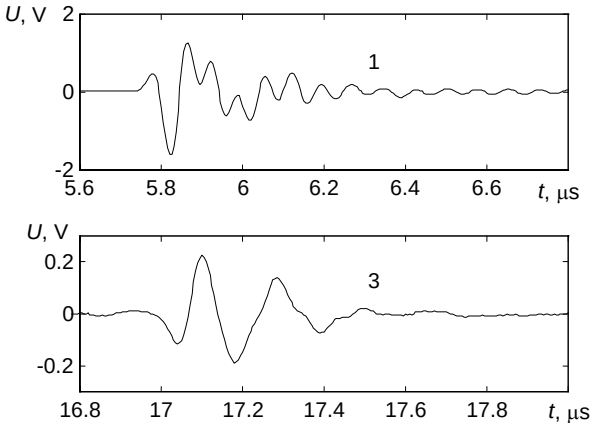

Fig.5. Only three reflections are observed in blood

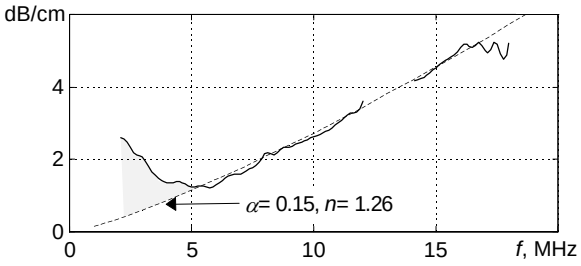

Fig.6. Ultrasound attenuation versus frequency in blood

absorption in biologic liquids versus frequency in the frequency range up to 17 MHz. We have obtained that the absorption in citrated non-clotted blood is described by the dependence $\alpha=0.15 \cdot f^{1.26}$, what is in a good correspondence with the depedencies $\alpha=0.15 \cdot f^{1.21}$ and $\alpha=1.15 \cdot f^{1.28}$ obtained by Narayna et al.[1] and Carstensen et al.[6].

References

1. **Narayana P. A. , Jonatan Ophir, Maklad N. F.** The attenuation of ultrasound in biological fluids. J.Acoust. Soc. Amer., vol. 76, No 1, pp. 1-4, 1984.
2. **Kremkau F.W.** Biomolecular absorption of ultrasound. III. Solvent interactions. J. Acoust. Soc. Amer., vol. 83, No 6, pp. 2410-2415, 1988.
3. **Choi P.-K., Bae J.-R., Takagi K.** Ultrasonic spectroscopy in bovine serum albumin solutions. J. Acoust. Soc. Amer. , vol. 87, No 2, pp. 874-881, 1990.
4. **Rokhlin S. I. , Lewis D.K, Graff K. F. , Laszlo Adler.** Real- time study of frequency dependence of attenuation and velocity of ultrasonic waves during the curing reaction of epoxy resin. J. Acoust. Soc. Amer. , Vol. 79, No 6, pp. 1786-1793, 1986.
5. **Challis R. E., Harrison J.A., Holmes A.K., Cocker R. P.** A wide bandwidth spectrometer for rapid ultrasonic absorption measurements in liquids. J. Acoust. Soc. Amer. , vol. 90, No 2, pp. 730-740, 1991.
6. **Carstensen E.L., Schwan H.P**. Absorption of sound arising from the presence of intact cells in blood. J.Acoust.Soc.Amer. Vol.31, p.185-189, 1959.

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Ultragarsinës spektroskopijos metodas biologinëms terpëms tirti

Reziumë

Nagrinëjama galimybës taikyti ultragarsiná spektrometriná metodà biologinëms terpëms tirti. Pasiûlytasis metodas remiasi plaèiajuosèiø ultragarsiniø signalø daþninës slopinimo priklausomybës matavimu. Apraðoma metodo realizacija. Pateikiami matavimø etaloninëje aplinkoje ir kraujyje rezultatai.

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