LETTERS TO THE EDITOR

Comparison of thermodynamic and finite amplitude methods of B/A measurement in biological materials

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The nonlinearity parameter B/A has been determined for various biological solutions and soft tissues using both the thermodynamic and finite amplitude methods. This letter is to show that agreement achieved between the two methods is within a fraction of a percent for liquid samples and within 10% for the soft tissues studied.

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INTRODUCTION

This Letter reports agreement between the finite amplitude and the thermodynamic methods for determining the nonlinearity parameter B/A of biological materials. Presentation of the complete details of the methodology and results of measurements will follow soon.

Measurements of the nonlinearity parameter B/A of solutions of biological macromolecules and of excised tissues by the finite amplitude method have been reported.[1-3] Therein the B/A value for protein solutions was found to increase linearly with solute concentration but, for a fixed concentration, solutions of dextran were shown to be relatively insensitive to the molecular weight of the solute molecules. Whole mammalian blood, whose dry weight consists largely of protein, exhibited a B/A value essentially that of protein solutions of the same dry weight concentration. Excised mammalian liver yielded a B/A value larger than blood, but homogenization of the tissue reduced this B/A value.

The finite amplitude method was employed for these measurements because of its potential to be used for B/A measurements *in vivo*. Because the calibration procedures for the finite amplitude method are rather complex, it was important to confirm the observations using a completely different method. Furthermore, in the measurement of soft tissues with the finite amplitude method, the inhomogeneous nature of the medium and the attendant phase cancellation effects could introduce errors in the B/A measurement procedures which would not be easily detected.

The thermodynamic method[4,5] of B/A measurement relies on measuring directly the change of sound velocity with hydrostatic pressure and temperature. Since the velocity, instead of the amplitude of the sound wave, is measured the method does not require the calibration of transducers and is free from phase cancellation effects. Moreover, since sound velocity, hydrostatic pressure, and temperature can all be measured to high degrees of accuracy, the thermodynamic method is potentially very accurate.

I. METHODS OF MEASUREMENT

The nonlinearity parameter B/A, through thermodynamic expansion, can be expressed in the sum of two terms[4]; a pressure derivative term $(B/A)'$, which is the dominant part of the expression, and a temperature derivative term $(B/A)''$, as

$$\frac{B}{A}=\rho_0 c_0\left(\frac{\partial c}{\partial p}\right)_T+\frac{\beta T\rho_0}{c_p}\left(\frac{\partial c}{\partial T}\right)_p=\left(\frac{B}{A}\right)'+\left(\frac{B}{A}\right)''. \quad (1)$$

Here, ρ_0 is the undisturbed density, c_0 is the infinitesimal wave velocity, $(\partial c/\partial p)_T$ is the change of sound speed with pressure at constant temperature, β is the volume coefficient of thermal expansion, T is the absolute temperature, c_p is the specific heat at constant pressure, and $(\partial c/\partial T)_p$ is the change of sound speed with temperature at constant pressure. The method of measurement based on Eq. (1) is often referred to as the thermodynamic method, and was first suggested by Coppens *et al.*[5] for measurement of liquids. To accommodate the special nature of biological specimens, of interest in the present study, the measurement apparatus including the velocimeter and electronics was modified from that first described by these investigators.

The instrumentation is comprised of a velocimeter completely enclosed in a water-filled pressure vessel, a hydraulic pump to vary the hydrostatic pressure inside the pressure vessel through a nickel bellows, within a range of pressure between atmospheric and 2000 psi, a Bourdon tube-type pressure gauge to measure the pressure to $\pm 0.1\%$, and a temperature controlled water bath for maintaining the velocimeter at the desired temperature to ± 0.002 °C. Appropriate electronic instrumentation is employed for the precise measurement of the propagation time of an acoustic pulse traveling through the sample in the velocimeter.

The velocimeter consists of a sample chamber made of type 304 stainless steel whose ends are parallel and concentrically mounted quartz crystal transducers resonant at 3 MHz. The separation of the crystals was measured to be

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TABLE I. Direct comparison of B/A values by the thermodynamic and finite amplitude methods.

Material	ρ g/cc	c m/s	β $°C^{-1}$	c_p $J/(g\text{-}°C)$	$(\partial c/\partial p)_T$ $m/(s\text{-psi})$	$(\partial c/\partial T)_p$ $m/(s\text{-}°C)$	$(B/A)'$	$(B/A)''$	B/A (thermodynamic)	B/A (finite amplitude)
Water	0.996	1509	3.67×10^{-4}	4.18	0.0119	1.748	5.17	0.14	5.31	5.3
BSA (38.9%)	1.094	1615	3.67×10^{-4}	4.18	0.0130	1.115	6.58	0.10	6.68	6.64
Beef liver	1.05	1590	3.67×10^{-4}	4.18	0.0140	1.15	6.78	0.10	6.88	6.42

3 ± 0.003 in. with a cathetometer, and the change in length of the sample chamber at different pressures and temperatures is corrected by using the thermal coefficient of expansion, the elastic tension moduli, and Poisson's ratio as specified by the manufacturer (private communication). To measure the time of flight of an acoustic pulse traveling through a sample in the velocimeter, the received acoustic signal, together with the electromagentic pickup of the electrical driving signal to the source, is displayed on one channel of an oscilloscope, which has a bandwidth of 100 MHz and a time resolution of 1 ns. The second channel displays a series of time marks which are synchronous with the driving signal applied to the source. The interval between time markers is adjustable and set to $\pm 0.001\%$ with a digital counter. By using the delayed sweep option of the oscilloscope, which allows the expanded display of a small portion of the waveform, the time markers can be aligned to a fixed reference point of the wave packet to the accuracy of 1/100 of a cycle, thus providing for 1 part in 10^4 resolution in the measurement of the time of flight.

In the computation of B/A using Eq. (1), both the absolute velocity in the sample and change of velocity with applied pressure or temperature are required. The time-of-flight measurement described above provides adequate resolution for measuring the small change of velocity, but must be corrected for a fixed time delay associated with the electrical circuits and the transducers in order to measure the absolute velocity accurately. Water was used as a reference liquid to determine the delay due to the electronics.[6] Results of measurements indicate that the delay amounts to approximately a 1% correction to the absolute velocity, and does not change with pressure or temperature applied to the velocimeter.

The finite amplitude method used in this comparison has been described in detail in a previous paper[2]; it is based on the method of Adler and Hiedemann,[7] but with added consideration for diffraction and absorption. The method is suitable for measuring biological materials with near linear frequency dependence of absorption. Specifically, $(\alpha_2 - 2\alpha_1)x$ should be smaller than $\frac{1}{2}$, where α_1 and α_2 are, respectively, the fundamental and second harmonic frequency absorption coefficients and x is the distance between the sound source and the observation point along the wave propagation path.

II. SAMPLE PREPARATION

Liquid samples were prepared in a similar manner as described in previous papers.[2] Tissue samples were obtained fresh from the slaughter house, stored at 5 °C, and studied within 24 h. They were sculpted into the cylindrical shape, with parallel end surfaces, about 3 in. long and 2 in. in diameter, to conform to the shape of the sample chamber. After mounting the tissue sample, 0.9% saline was introduced to fill completely the sample chamber.

III. RESULTS AND DISCUSSION

Direct comparison of B/A values determined by the thermodynamic method and the finite amplitude method were made for degassed, distilled water, bovine serum albumin (BSA) solutions, and whole beef liver. The results and the parameters used for computations are summarized in Table I. The β and c_p values for water were obtained from the literature.[8] For the solutions and tissues measured, values of β and c_p were not available. However, the c_p value for rabbit liver has been reported[9] to be about 20% less than that of water, and a β value for a 30% BSA solution was determined to be 13% greater than water, using a calibrated capillary to determine the expansion of a known sample contained in the volumetric flask. Based on these, it was assumed that β and c_p values for the biological materials measured were not significantly different from those of water, for the purpose of the computation of B/A, since β and c_p are involved only in the $(B/A)''$ term which contributes only about 3% to the B/A value. The overall accuracy of the thermodynamic method is estimated to be $\pm 3\%$ for liquids, and $\pm 5\%$ for tissues.

In order to reduce the influence of sample variation in the comparison of the finite amplitude and thermodynamic methods, measurements were made on the same sample of BSA solutions and on samples of beef liver obtained from the same organ specimen. The result of this direct comparison is shown in Table I where the two techniques are seen to agree within a fraction of a percent for liquid samples, but a 7% discrepancy is observed for the beef liver sample, which may be related to gas content associated with autolysis or phase cancellation effects associated with the finite amplitude method.

IV. CONCLUDING REMARKS

Thus the finite amplitude method of B/A measurement provides values in excellent agreement with the thermodynamic method in degassed, distilled water, BSA solutions, and liver *in vitro*. This gives credence to its use in *in vivo* measurement for more complete characterization of tissues in normal and diseased states.

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Bone-conduction measurement and calibration using the cancellation method

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We have demonstrated that the cancellation method can be used for calibrating the bone-conduction vibrator in relation to the headphone of the audiometer and for an accurate measurement of a maximum bilateral conductive hearing loss. It is discussed how the correctness of the adjustment for the cancellation can be verified by recording brainstem auditory evoked potentials.

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The effective abilities of the cancellation method have been shown by Bekesy (1932) to prove psychophysically that in the perception of the bone-conduction sound stimuli the inner ear is involved. By measuring the electric response of the cochlea for air and bone conducted stimuli Lowy (1942) proved, using the cancellation method, that the cochlea is stimulated in both situations in the same manner.

An audible pure tone presented by headphone to one ear can be cancelled by offering a tone to the same ear using the bone-conduction vibrator. To achieve this cancellation the frequencies of both pure tones have to be exactly the same, and the amplitudes as well as the phase relation between the signals have to be adjusted very accurately. When a signal, in our case a sinusoid, is presented to one detector by two different transducers along different pathways, the detector is stimulated by a superposition of both versions. In formula:

$$I(s) = A \sin \omega t + B \sin(\omega t + \phi) .$$

A presentation of the calculated stimulus is shown in Fig. 1. When both sinusoids arrive at the detector in phase and with equal amplitude the result is a pure addition. When the signals arrive with equal amplitude but in opposite phase the result is zero signal, i.e., both sinusoids cancel each other. The shape of the curve calculated is very steep. When the two signals have exactly the same stimulation intensity, a small phase shift around the 180° stimulation implies a sharp decrease of the attenuation. The cross section of Fig. 1 shows how a difference in amplitude of only 2 dB affects the course considerably, especially in the area around the phase shift of 180°. In that case the calculated maximum attenuation is seen to be about 20 dB instead of a complete cancellation. This attenuation reduces rapidly by increasing the difference between the amplitudes. Theoretically cancellation is a very sensitive method for relating the intensity of two signals using the human ear, with its excellent dynamic range, as a zero-detection instrument.

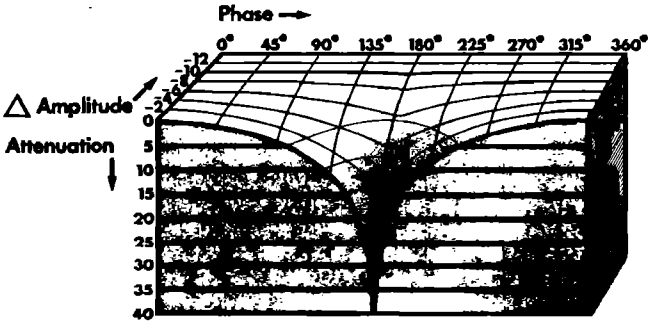

FIG. 1. The calculated effect of cancellation.