

DEPENDENCE OF THE ULTRASONIC NONLINEARITY PARAMETER
B/A ON CELLULAR-LEVEL STRUCTURE

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Abstract

The nonlinearity parameter B/A of biological media is considered useful as a tissue characterization parameter and as an accurate model of finite amplitude wave propagation in such applications as ultrasonic hyperthermia and lithotripsy. Some attention has been devoted to the influence of the structure of the media on the B/A. The dependence of B/A on cellular-level structure has been investigated by the thermodynamic method using liposomes as a cell model. It was found that B/A increases considerably near the transition temperature of the liposomes, suggesting that the nonlinearity parameter reflects the state of the liposome membrane.

I. Introduction

The nonlinearity parameter B/A of biological media has been actively investigated during the past several years [1-5]. Based on a number of studies of the B/A parameter on both model biological media and biological media, a description is emerging. Modeling biological media as a mixture of a number of tissue constituents, has suggested that the B/A of tissue may be the linear combination of the constituents' B/A values [6,7]. Earlier studies [3] also provided evidence suggesting that B/A depends not only on the relative concentration of each constituent, but also upon the way that each constituent is organized to form the medium, i.e., the structure. These studies have encouraged further inquiry into how the B/A value depends upon the structure of a medium. Herein, results of studies at the cellular level are presented and some implications are discussed.

II. Measurement System and Its Testing

B/A can be expressed in terms of the derivative of velocity with respect to pressure during an adiabatic process as

$$B/A = 2\rho_0 c_0 (\partial c / \partial p)_s$$

where ρ_0 is ambient density of the medium,

and c_0 is the ambient infinitesimal speed of sound [9]. A B/A measurement system has been developed using the pressure jump scheme [5] to create an adiabatic pressure change and employing a cross-correlation method to determine the time delay between a pulse transmitted through a sample and a reference pulse, and, thereby, the speed of wave propagation.

Figure 1 is a block diagram of the system. A 20 cycle gated cw burst at 3 MHz is generated by a Wavetek 271 pulse generator, which is then divided by a power splitter into two equal amplitude pulse. One pulse provides a reference after being delayed acoustically. The acoustic delay line is composed of two transducers separated by a column of water, and provides a delay of about 50 μ sec. The other pulse is amplified to drive one of the transducers of the velocimeter. The velocimeter is composed of two transducers, parallel and coaxial to each other spaced about 7.6 cm apart, to contain the sample [3]. The unit can be pressurized and depressurized hydraulically. The reference pulse, after the acoustic delay, and the pulse after passing through the sample in the velocimeter, are captured by a Tektronix 2430A digital storage oscilloscope which has two channels and a record length of 1 Kbyte. It is set to digitize both channels at 50 megasamples per second simultaneously and the digitized waveform can be transferred via an IEEE 488 interface to an AT&T PC for signal processing. A Neslab 500DD water bath is used to maintain the temperature of both the velocimeter (with sample) and the acoustic delay to $\pm 0.01^\circ\text{C}$. The temperature of the water bath is measured with a Fluke 2180A (not shown) digital thermometer.

A cross-correlation method is used to calculate the relative time delay between two pulses. In order to determine the time delay accurately, both frequency domain zero padding interpolation and second order parabolic curve fitting interpolation were performed to reduce uncertainty in delay measurement to ± 1.5 ns. The total time delay between the transmitted pulse and the received pulse is the sum of the

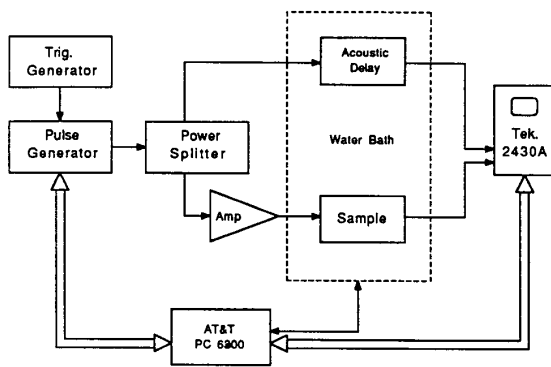


Fig. 1 Block Diagram of the B/A measurement System

time delay created by the acoustic delay and the relative delay measured by the digital oscilloscope. The total time delay is calibrated against known velocity values for degassed distilled water and is used to calculate the propagation velocity in a sample. The uncertainty of the relative velocity measurement was determined to be ± 2 cm/sec and that of the absolute velocity measurement was determined to be $\pm 0.013\%$. Figure 2 shows a typical plot of the sound speed versus time.

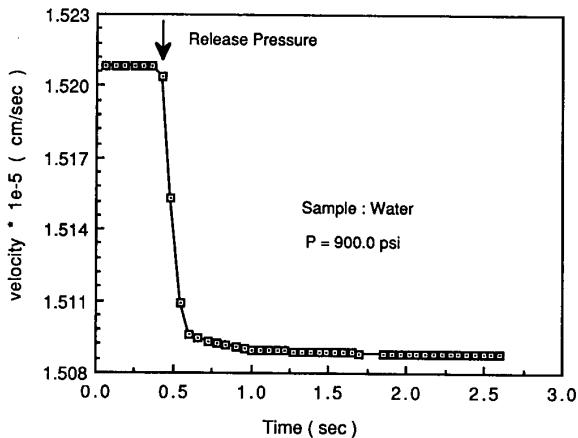


Fig. 2 Velocity Change During Pressure Jump

The advantage of using the pressure jump method over more traditional thermodynamic methods is the decrease in time required to obtain B/A data. Cross-correlation of two pulses to determine the relative time delay takes advantage of high signal to noise ratio, as correlation rejects uncorrelated noise. Pulse operation avoids problems associated with standing waves.

The system was tested using three samples and found to be in good agreement with the B/A values reported in the literature, as shown in Table 1.

Table 1 Comparison of Measured B/A Values with the Literature Values

	present study mean \pm S.D.	Beyer, 1959	Law, 1981	Cobb, 1982
Degassed, Distilled water, T = 30 C	5.14 \pm 0.001	5.22	5.31	5.12
Degassed, Distilled water, T = 40 C	5.36 \pm 0.007	5.38		
25% Dextrose Solution T = 30 C	6.13 \pm 0.01		5.95	
Ethylene Glycol, T = 30 C	9.82 \pm 0.006	9.7		9.64

III. Materials

Liposomes, small vesicles with diameters between 0.02 to 10 μ m whose membranes consist of a bilayer of phospholipid molecules, provide a model for the biological cell membrane. As temperature is increased the membrane goes through a phase transition from an ordered crystalline-like state below the transition temperature to a fluid-like state above it. Liposomes with three different structures were studied. The small unilamellar vesicles (SUV) have diameters between 20 and 50 nm. The large unilamellar vesicles (LUV) range from about 70 to 400 nm in diameter. The largest liposomes are multilamellar vesicles (MLV) which, rather than being single bilayers, consist of concentric spheres of bilayers. They exhibit wide size distribution in the range of 0.1 to 10 μ m. All the liposomes studied are prepared from a 4:1 (w/w) ratio of dipalmitoyl phosphatidyl choline (DPPC) and dipalmitoyl phosphatidyl glycerol (DPPG) phospholipid. The liposomes were suspended in HEPES buffered saline (10 mM HEPES, 139 mM NaCl, 6 mM KCl, pH.7.4).

IV. Results and Discussion

Figure 3 shows B/A in LUV liposome suspensions of two different concentrations, as a function of temperature. The LUV liposomes of this comparison are known to have its transition temperature at 42.5 $^{\circ}$ [9], where the B/A value reaches its maximum value. Figure 4 shows B/A for MLV and SUV liposomes suspensions. Despite variations in concentration, a dramatic difference is seen between B/A in SUV and MLV suspensions. B/A in SUV has a broader peak and lower transition temperature, while B/A in MLV has a sharp peak, higher transition temperature. Similar behavior has been observed for acoustic absorption in the liposome suspension of the same lipid composition [10]. The data also exhibit a

region just beyond the transition temperature where the B/A value of the suspension is even lower than the buffered saline.

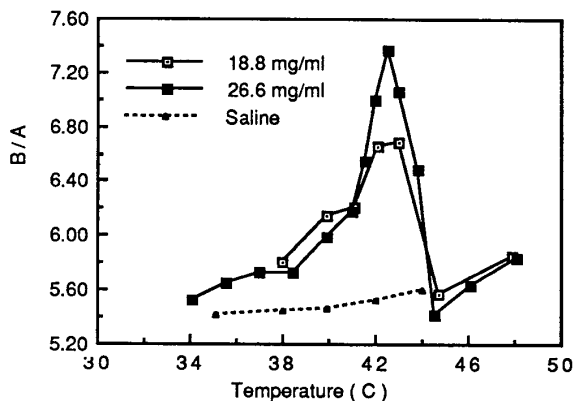


Fig. 3 B/A in LUV Liposome Suspension

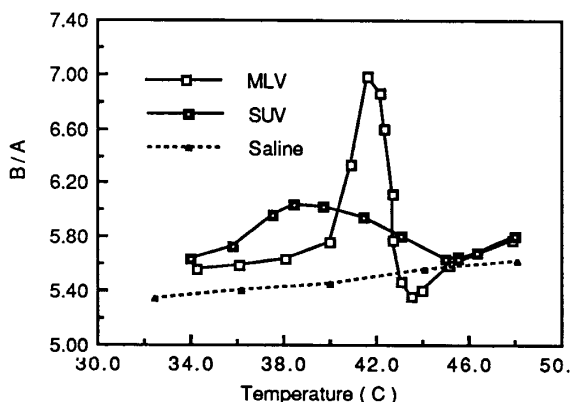


Fig. 4 B/A in MLV and SUV Liposome Suspension

In order to determine the relative contribution of each component to the B/A value of the suspension, it was assumed that the B/A value of the liposome suspension can be represented as a linear combination of the B/A values of the lipid bilayer and of the buffered saline in which the liposomes are suspended, with percentage weight concentration as the coefficients. That is

$$(B/A)_{\text{susp.}} = C_{\text{lip.}}(B/A)_{\text{lip.}} + C_{\text{buffer}}(B/A)_{\text{buffer}}$$

Figures 5 and 6 show the data of Figures 3 and 4, respectively, replotted but exhibiting the contribution due to the lipid bilayer only. The following are some observations: (1) It can be seen from the data that in the regions well below and well above transition, B/A exhibits values

of about 10, which is very close to the B/A value obtained for fat [3]. This result is expected since both fat and phospholipids have similar molecular structures [11]. (2) It is also observed that in the transition region the B/A value of the bilayer has a large value, approximately 10 times that of measured for parenchymal tissues. (3) Even if the concentration is normalized, SUV and MLV's still show differing B/A profiles with this difference possibly being due to the difference in the way the phospholipids are organized to form vesicles, viz., differences in structure. (4) At least in the case of the LUV and MLV's, there exists a region just beyond transition, in which lipid bilayers appear to exhibit zero nonlinearity.

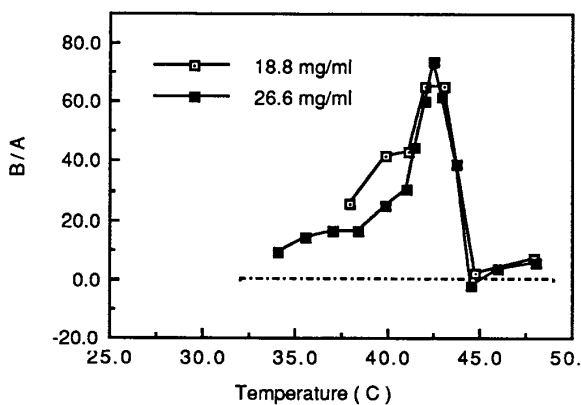


Fig. 5 B/A of Lipid Bilayer in LUV

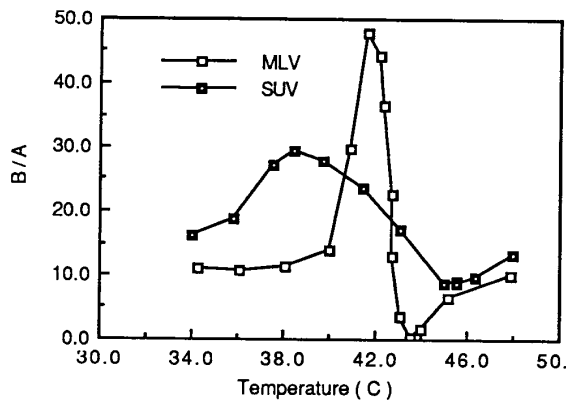


Fig. 6 B/A of Lipid Bilayer in MLV & SUV

Acknowledgements

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VI. References

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