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ACOUSTIC NONLINEARITY PARAMETER B/A OF BIOLOGICAL MEDIA

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INTRODUCTION

Considerable work, both theoretical and experimental, has been reported over the last 10 years on the propagation of finite amplitude ultrasound in liquid media of biological significance, and in biological tissue. These studies, in particular inspired by increasing medical application of focused ultrasound [1,2] by the development in acoustic microscopy [42], have contributed towards a heightened awareness of the potential role of finite-amplitude wave effects in diagnostic as well as in therapeutic ultrasound. Until nearly 1980 the propagation of ultrasound through biological media has been modelled as a linear process where changes in density, temperature etc. during compression and rarefaction phases of an ultrasonic wave propagating through the media were assumed to take place linearly with the pressure, and where absorption was assumed to be independent of the ultrasonic intensity [3]. About 10 years ago doubt was thrown on the validity of the linear assumption [4,5] and evidence was created of intensity dependent absorption in biological tissue [6] due to harmonic amplitude generation in focused ultrasonic fields caused in particular by convective nonlinear contributions.

On background of the extensive and still increasing use of ultrasound for diagnostic as well as for therapeutic purposes around the world these findings started a search for nonlinear ultrasonic effects in biological media in laboratories in several countries.

In ultrasonic imaging systems a trend over the years seems to have been the use of still increasing intensities to gain better signal-to-noise ratios. Temporal peak intensities in excess of 1000 W/cm^2 are not uncommon in fields of focused sources. Moreover, the high intensities commonly used in ultrasonic surgery and therapy make likely the existence of nonlinear ultrasonic propagation effects in tissue. These effects, being of a cumulative character, comprise for instance wave distortion, formation of higher harmonics, finite-amplitude absorption effects and probably also acoustic saturation which will limit the ultrasonic energy transfer over a known distance from the source and into the biological medium. The use of focused ultrasound makes it possible to obtain larger ultrasonic intensities in the focal region before shock formation, than it is possible, when using plane waves, and the reduced heat diffusion by unfocused ultrasound leads to a lower threshold for lesion formation than found by focused ultrasound [7].

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A better understanding of the nonlinear interaction of ultrasound with biological media was felt as a need, but first information about the nonlinear interaction of ultrasound with biological media was felt as a need, but first information about the nonlinear character of biological media had to be obtained, including estimates of the magnitude of acoustic nonlinearity to be expected to be found in these media. Studies performed over the last 10 years have yielded some evidence, - but still not enough - that the material nonlinearity of biological media represented by their second-order ultrasonic nonlinearity parameter B/A may qualify as a *tissue characterizing parameter* on a par with attenuation, absorption, scattering, ultrasonic velocity and impedance. A critical review of the most essential contributions to the development throughout the last 10 years in procedures B/A of biological media will be presented in this paper.

Theoretical foundations.

By tissue characterization using material nonlinearity we will understand the identification of a physical parameter - here the nonlinearity parameter B/A - of a small volume of the tissue, which is so well correlated with the condition of the tissue, that the measurement of this parameter may be used as a reliable and reproducible index of the pathological condition of the tissue volume investigated.

The second-order nonlinearity parameter B/A, which may qualify according to the above definition, is obtained through a Taylor series expansion of the equation of state for adiabatic changes in a fluid expressed by:

$$p = p_o + \rho_o \left(\frac{\partial p}{\partial \rho}\right)_{o,s} \left(\frac{\rho - \rho_o}{\rho_o}\right) + \frac{\rho^2}{2} \left(\frac{\partial^2 p}{\partial \rho^2}\right)_{o,s} (\rho - \rho_o)^2 + \ldots \qquad (1)$$

where p and p_o are the instantaneous and the hydrostatic pressure, respectively, ρ denotes the density of the medium and the subscripts o and s denote the partial derivatives at equilibrium density ($\rho = \rho_o$) and at constant entropy s. Equation (I) may be rewritten as:

$$p - p_o = A \left(\frac{\rho - \rho_o}{\rho_o}\right) + \frac{B}{2} (\rho - \rho_o)^2 + \ldots \qquad (2)$$

where

$$A = \rho_o \left(\frac{\partial p}{\partial \rho}\right)_{a,S} = \rho_o c_o^2 \qquad B = \rho_O^2 \left(\frac{\partial^2 p}{\partial \rho^2}\right)_{a,S} = 2\rho_o^2 c_o^3 \left(\frac{\partial c}{\partial p}\right)_{a,S}$$

where c_o is the velocity of sound.
From (2) the ratio B/A may be found using simple thermodynamical relations [8] as:

$$\frac{B}{A} = 2\rho_o c_o \left(\frac{\partial c}{\partial p}\right)_{a,S} = 2\rho_o c_o \left(\frac{\partial c}{\partial p}\right)_T + \frac{2c_o T\,\beta}{c_p}\left(\frac{\partial c}{\partial T}\right)_p = \left(\frac{B}{A}\right)' + \left(\frac{B}{A}\right)'' \qquad (3)$$

where β is the volume coefficient of thermal expansion, T is the absolute temperature, c_p is the heat capacity at constant pressure and where the derivatives of the velocity of sound with respect to pressure and temperature are taken at constant temperature and at constant pressure, respectively. The first term of (3) shows B/A's relation to the pressure-dependence of the material's stiffness.

A close relationship exists between the nonlinearity parameter B/A and the molecular structure of certain liquids. This relationship may for instance be expressed by [9]:

$$\frac{B}{A} = 2\,\gamma\,K_2 + 2\,K_1\,(\gamma - 1) \qquad (4)$$

where K_1 and K_2 are Rao's constant and Carnevale and Litovitz constant, respectively, which express reactions between the derivatives of the velocity of sound and the density with respect to temperature and pressure [10, 11].

Several authors have discussed the close ties between B/A and the intermolecular or interatomic potential [12]. According to their theories, aggregates of biological cells, being much larger than the intermolecular scale, should not have a great influence on the magnitude of B/A. However, particular efforts have recently been made to develop models for prediction of effective B/A-values of immiscible liquid mixtures and tissue [13, 14]. The effective nonlinear parameter B/A of a mixture was found as:

$$\left(\frac{B}{A}\right)_{eff} = \frac{1}{\zeta^{2}_{eff}} \sum_{l=1}^{n} \zeta_l^2 \left(\frac{B}{A}\right)_l x_l \qquad (5)$$

where the effective adiabatic compressibility

$$\zeta_{eff} = \frac{1}{\rho\, c^2} = \sum_{l=1}^{n} \zeta_l\, x_l \qquad \sum_{l=1}^{n} x_l = 1.$$

x_l are the volume fractions and n is the number of different components that are sufficient to describe the liquid mixture or tissue. But more research is needed to verify the applicability of these models for determination of B/A of complex materials like tissue and in order to prove the significance of B/A as an individual tissue characterizing parameter. The magnitude of the B/A-ratio, which for most biological media is between 5 and 11, signify the ability by the media of forming higher harmonics to an original sinusoidal wave of finite-amplitude during its propagation away from the wave source. This cumulative distortion course may for high source amplitudes in low-loss media lead to formation of weak shock waves [15]. The increased absorption on the produced higher harmonics reduces the wave front steepness and finally the wave profile returns to its original sinusoidal shape, but with an infinitesimal amplitude. As biological tissue is an example of a highly lossy medium, where the attenuation does not increase with l and l.3 [16], the finite-amplitude distortion and attenuation course to take place in tissue demands a high ultrasonic source level in spite of the magnitude of the B/A-values found in tissues. An algorithm for calculation of the generation of harmonics and of their attenuation in biological tissue has been developed [17], and a theoretical parameter study of nonlinear effects
when a focused ultrasonic beam is absorbed in a tissue-like medium has recently been published [33] which suggests the usefulness of nonlinear effects in selectively increasing the temperature at depth in tissue.

Methods of measurement of B/A.

Among many well developed procedures for determination of B/A of pure liquids, two have, in particular, found application by measurements done on biological media. These two procedures are (1) the finite-amplitude method and (2) the thermodynamic method. These two fundamental procedures have been used in several modified versions for determination of B/A, and their advantages, disadvantages and accuracy of data produced will be discussed in subsequent paragraphs. Moreover, a new and promising procedure, (3) the real-time non-linear parameter tomography, has been suggested and has been studied in some detail [18, 19, 20].

1. The finite-amplitude method.

Based on Fubini's [21] Fourier series solution to the exact fundamental equations for finite-amplitude wave propagation through a lossless fluid the following simple expression for the

second harmonic pressure amplitude p_2 as a function of dimensionless source distance may be found:

$$p_2 = p_s \frac{J_n(2\delta)}{\delta} \tag{6}$$

where p_s denotes the pressure amplitude at the source and where $\delta = x/l$, with l being the so-called discontinuity distance related to B/A through the expression:

$$l = \frac{\rho_o c_o^3}{\pi f \left(2+\frac{B}{A}\right) p_s} \tag{7}$$

Expansion of the Bessel function $J_n(2\delta)$ in (6) in a power series, retaining only the first and the second term leads to the following expression for p_2 as a function of source distance x.

$$\left(\frac{p_2(x)}{x\, p_s^2}\right)_{xp_s^2 \to o} = \frac{\left(2 + \frac{B}{A}\right) \pi f}{2R_o c_o^3} \tag{8}$$

From (8) B/A may be found plotting the measured values of the ratio $p_2(x)/(x\, p_s^2)$ as a function of extrapolating back to the source ($x = 0$). This approximation is accurate to within 2% for $\delta < 0.25$ [22, 23].

Results are reported in [22] and [23] based on the use of (8) for determination of B/A in bovine serum albumin, hemoglobin from porcine blood and in porcine whole blood versus concentration. Experimental data showed that B/A of bovine serum albumin is an approximately linear function of concentration (i.e. dry weight content), while B/A exhibits virtually no dependence on solute molecular weight of the organic materials studied.

Several assumptions underlie the development of (8). They are, plane wave propagation, source distance much smaller than l and no influence of loss effects. The last assumption is most critical and limiting to the applicability of (8), due to the high losses of ultrasonic energy normally found, in for instance tissue. A further development of (8) was made [23] based upon the assumptions [24], that the attenuation of the fundamental and its second harmonic amplitudes are mutually independent, and that the rate of change with propagation distance of the second harmonic amplitude is the sum of changes caused by the nonlinear generation of the second harmonic and by its attenuation. Introduction of

these assumptions an expression somewhat similar to (8), but taking into consideration the attenuation effects, may be derived.

$$\left(\frac{p_2(x)}{x\, p_S^2}\right)_{xp_S^2 \to 0} = \frac{(2 + \frac{B}{A})\, \pi\, f}{2R_o c_o^3}\, e^{-(\alpha_1 + \frac{\alpha_2}{2})\, x} \qquad (9)$$

where α_1 and α_2 denote the attenuation coefficients for the fundamental and its second harmonic amplitudes.

Determination of B/A using (9) has been reported in [23] for homogenized as well as for whole liver.

Experimental determination of B/A using a finite-amplitude method like the one expressed by (9) will normally involve the use of two ultrasonic piston transducers positioned coaxially and with a few centimeters of distance between the transducer surfaces. This distance is occupied partly or fully by the biological medium to be investigated. Due to the short source-receiver separation distance influence of diffraction effects, as well as phase cancellations over the receiver surface, shall be expected [25, 26, 27], and corrections have to be introduced accordingly. After introduction of a diffraction correction term (9) should be accurate to within $\pm 2\%$ for $(\alpha_2 - 2\,\alpha_1)x < 0.5$ [28].

Errors in using the finite-amplitude method for experimental determination of B/A most arise from transducer calibration procedures. An estimated systematic error of $\pm 8\%$ for liquid for liquid and tissue samples studied is reported in [28].

The influence of diffraction as well as attenuation effects on the experimental measurements was taken into account in a semi-empirical finite-amplitude method [29] where the growth of the second harmonic amplitude over the source-receiver separation distance was measured using a piston receiver situated coaxially with, and being of the same size as, the piston source. A theoretical expression developed for the average pressure amplitude of the second harmonic to be measured at source distance x is given by:

$$|\langle p_2(r,x) \rangle| = \frac{(2 + \frac{B}{A})\pi f}{2\rho_o c_o^3} (I_1 - I_2) \qquad (10)$$

where I_1 is the absorption term given in (9) and where I_2 is an integral accounting for the diffraction effects and for phase cancellations across the receiver surface. In order to determine B/A, calculated values based upon (10) were compared with measured average pressure amplitude data. For several non-biological liquids studied, a measurement accuracy of ±4% using (10) was assumed.

The use of an acoustic microscope as a tool for nonlinear characterization of biological media was studied in [42], based on measurements of the second harmonic signal generated in a sample located at the focus of an acoustic lens.

2. The thermodynamic method.

A method for determination of B/A which does not require the use of finite-amplitude waves is based on the application of expression (3). In (3) the most crucial factors are the derivatives of the velocity of sound with respect to pressure and temperature for constant temperature and pressure, respectively. Expression (3) has for many years been used for calculation of B/A of liquids [15] as well as B/A of inhomogeneous materials like water-saturated marine sediments [15] as well as B/A of inhomogeneous materials like water-saturated marine sediments [30]. The advantage of basing the determination of B/A on (3) instead of a finite-amplitude method is that (3) does not depend on the characteristics of the ultrasonic field. However, it requires an estimate of the thermodynamical parameters β and c_p which are not known with great precision for most soft tissues. The values of β and c_p for water have, however, been used as substitute for the actual values of the samples [31]. Moreover, it is recognized that the terms of (3) involving the influence of and c_p only contributes about 3% to the overall determination of B/A.

Several experimental procedures have been used in order to find the dependence of the velocity of sound c on pressure and temperature. The use of a velocimeter procedure is reported in [31] which leads to an overall estimated accuracy of the thermodynamic method to be ±3% for liquids and ±5% for tissues, an accuracy also comprising the influence of sample length uncertainty and of inhomogeneity of the tissue which may lead to variations in velocity of sound across the path of propagation through the sample.

Measurement of phase changes caused by variations in the physical properties of the

medium forming the ultrasound propagation path may lead to rather accurate data for the variation in velocity of sound. A modified thermodynamic technique using the adiabatic relation between the velocity of sound and the pressure given in expression (3) has been reported in [32]. In this technique B/A is determined by measurement of changes in the travel time of ultrasonic tone bursts caused by changes in the ambient pressure. Travel time differences are measured by comparison between the phase of the tone burst and the phase of a reference signal. An accuracy of the order of ±5% for the B/A measurements in biological liquids has been found.

A modified version of the phase comparison method reported in [32] is formed by a continuous wave method which is applicable to attenuating media like tissue and which dos not require knowledge of the thermodynamic parameters β and c_p [33]. Based upon the relation between B/A and the change in sound velocity with the isentropic change in hydrostatic pressure in the biological medium studied, a relation between the change in phase ϕ per unit change in hydrostatic pressure and B/A may be developed as:

$$\frac{B}{A} = - \frac{2 \rho_o c_o^3}{L \omega} \left(\frac{\partial \phi}{\partial p} \right)_S \qquad (11)$$

where L is the length of the medium under investigation and where ω is the continuous wave angular frequency. Based upon (11) and the equivalent expression for an isothermal process, B/A has been determined for normal as well as for malignant tissues in the temperature range 20° C to 37° C.

The availability of small amounts of biological samples have frequently caused problems for experimental determinations of B/A. However, most recently a thermodynamic method capable of determining the B/A value of a 4-ml sample volume with a measurement error less than 0.7% has been reported [44]. The method involves a procedure in which the static pressure of the sample is altered very fast, to approximate and adiabatic process, during
during which the ultrasonic velocity, to form a basis for the calculation of B/A, is measured.

A comparison of the B/A-values obtained using the finite-amplitude method and the thermodynamic method [28, 31] shows that the agreement between result arising from the two methods seems to be affected by the nature of the sample due to the fact that discrepancies appear to increase with the degree of tissue inhomogeneity. The averaged values of B/A determined by the two methods differs by 9% for liver samples, 2% for pig fat and 1% for homogenized liver. These results seem to indicate the significance of tissue structure and inhomogeneity on the measured B/A-values.

Recently, a comparative study has been performed [46] of the B/A values obtained in biological media using (a) a finite-amplitude insert-substitution method, taking into account sound attenuation of the samples and transducer diffraction, and (b) an improved thermodynamical method, based on measurements of phase shift in the acoustic wave due to the change of ambient pressure. The results given in [46] indicate that B/A not only depends on the temperature and the solution concentration, but are also closely related to the specimen structural complication as also found in [28] and [31].

The influences of the structural factors of biological media on the acoustic nonlinearity parameter B/A was reported most recently in a comprehensive work [45]. At tissue, cellular and at molecular levels the structural dependencies of B/A was studied using the finite-amplitude and the thermodynamic methods in procedures where the structural features of the specimens were altered, while the chemical composition was kept unchanged. It was found, that the relative contributions due to structural features in 26% at the tissue level, 20% at the cellular level and 15% at the macromolecular level.

The dependence of B/A on the molecular structural features has recently been studied [43] by ten amino acids and by six proteins, using high-precision velocimetry for measurements at elevated pressures and using a new differential method for calculation of the B/A specific increments that provides an accuracy of relative measurements of B/A better than 0.3%. The results in [43] showed that replacement of a single atomic group within a molecule may cause large changes in the B/A concentration increment, while other parameters of the solutions like sound velocity and density may not be sensitive to the replacement.

3. Real-time nonlinear parameter tomography.

One of the inherent disadvantages connected with the use of the finite-amplitude method and the thermodynamic method is, that these methods are based on measurements performed on samples of tissue, and they are as much more *in vitro* than *in vivo* methods.

Moreover the two methods are giving information on the average B/A of the sample investigated, and reduction in sample size will lead to a B/A measurement which may be characterized as a discrete point measurement. In order to exploit the potential use of the nonlinearity parameter B/A in diagnostics and in tissue characterization, and *in vivo* method, which is able to give a two-dimensional picture of the B/A-variation through tissue and between organs, should be developed. Some of these objectives seem to be fulfilled by a new system - the real-time nonlinear parameter tomography system - investigated by a Japanese group [18, 19, 35]. In this system, which is aiming at in vivo measurements, an impulsive and relatively high-power pumping wave is applied on the biological tissue from a direction perpendicular to the direction of propagation of a continuous, low-intensity

probing wave of high frequency. Studies of counter propagating pump and probe waves have also been investigated [35]. In this way the phase of the probing wave is modulated instantly by an expression involving the product of an *equivalent* nonlinearity parameter $(B/A)_e$ of the tissue and the pressure function of the pumping wave. The spatially modulated probing wave is then detected and demodulated to derive the distribution of B/A along the path of the probing beam. By shifting the position of the probing beam a two-dimensional image of the B/A variation may be obtained of the tissue investigated. The data for the system are: Probing wave: Pulsed operation with one period of a 500 kHz wave. Power, 19 mW/cm^2. Imagining area: 0.16 x 0.16 m^2. Resolution in two dimensions: 0.002 m.

This system, which looks promising due to its inherent advantages, has not yet been able to give any magnitude of the B/A-values of tissues to be compared with values found using other methods. A total separation of the nonlinear parameter from other factors influencing the contents and the quality of the images produced must primarily by obtained in order to develop a system which can produce reliable clinical information about B/A as a tissue characterizing factor.

Discussion and conclusions.

Several groups of scientist have during recent years contributed nonlinearity values B/A of various biological materials in order to create the necessary basis for a characterization of these materials. Some B/A-values for biological materials are given in table I.

From Table I. may be seen that the highest B/A-values are found in fat and fatty tissue. B/A-values up to 11 have been reported for pure pig fat, while human breast fat shows values between 9 and 10, depending on temperature. Increasing fat contents in tissue seems to lead to increasing values for B/A. This is supported by the mixture rules reported in [13] and [14].

The lower B/A values found in homogenized liver compared to whole liver makes it likely that homogenization destroys the nonlinearity-producing qualities of the liver structure.

The small deviations between B/A found for the same biological material using either the thermodynamic or the finite-amplitude method seem to support the statement that these two methods - in spite of their *in vitro* character - are now so refined that they can replace a one another.However, the scatter of the B/A -values measure, represented by the estimated uncertainties seems to indicate that improvements of the two experimental methods still need to be made in order to obtain reliable and reproducible B/A-data to characterize biological media.

Table 1 Some *B/A* values for biological materials

	Biological material (and state)	Method[a]	*B/A* (and uncertainty)	Reference
1.	Bovine serum albumin (BSA) (20 g/100 cm^3, 25°C)	Therm.	6.23 (± 0.25)	31
	BSA (22 g/100 cm^3, 30°C)	F.A.	6.45 (± 0.30)	21
	BSA (38.9 g/100 cm^3, 30°C)	F.A.	6.64	30
	BSA (38.9 g/100 cm^3, 30°C)	Therm.	6.68 (± 0.2)	30
2.	Haemoglobin (50%, 30°C)	F.A.	7.6	22
3.	Whole porcine blood (12% haemoglobin, 7% plasma proteins, 30°C)	F.A.	6.2 (± 0.25)	22
4.	Beef liver (Whole, 23°C)	F.A.	7.75 (± 0.4)	22
	Beef liver (Homogenized, 23°C)	F.A.	6.8 (± 0.4)	22
	Beef liver (Whole, 30°C)	F.A.	6.42	30
	Beef liver (Whole, 30°C)	Therm.	6.88	30
	Beef liver (Whole, 30°C)	Therm.	6.54 (± 0.2)	32
	Dog liver (30°C)	F.A.	7.6 – 7.9 (± 0.8)	35
	Pig liver (25°C)	F.A.	6.7 (± 1.5)	36
	Human liver (Normal, 30°C)	F.A.	7.6 (± 0.8)	35
	Human liver (Congested, 30°C)	F.A.	7.2 (± 0.7)	35
5.	Pig fat	Therm.	10.9	27
	Pig fat	F.A.	11.0–11.3	27
	Human breast fat (22°C)	Therm.	9.21	32
	Human breast fat (30°C)	Therm.	9.91	32
	Human breast fat (37°C)	Therm.	9.63	32
6.	Canine spleen	F.A.	6.8	37
	Dog spleen	F.A.	6.8 (± 0.7)	35
	Human spleen (Congested)	F.A.	7.8	37
	Human spleen (Normal, 30°C)	F.A.	7.8 (± 0.8)	35
7.	Beef brain (30°C)	F.A.	7.6	27
8.	Beef heart (30°C)	F.A.	6.8–7.4	27
9.	Pig muscle (30°C)	F.A.	7.5–8.1	27
	Pig muscle (25°C)	F.A.	6.5 (± 1.5)	36
10.	Dog kidney (Normal, 30°C)	F.A.	7.2 (± 0.7)	35
	Canine kidney (30°C)	F.A.	7.2	37
11.	Human multiple myeloma (22°C)	F.A.	5.6	32
	Human multiple myeloma (30°C)	F.A.	5.8	32
	Human multiple myeloma (37°C)	F.A.	6.2	32

[a]Therm. = thermodynamic method; F.A. = finite amplitude method

The temperature influence of B/A for various types of tissues, as for instance seen in relation to human multiple myeloma, may probably be related to the molecular structure. Theories for the molecular structure and the intermolecular potential as a prospective physical basis for the nonlinearity of a material was first put forward in [12]. A general validity of these theories will mean that structures formed by aggregates of biological cells in tissues, where a characteristic dimension is much larger than the intermolecular scale, should not contribute significantly to the magnitude of B/A. This statement seems to be opposed by the findings for whole and homogenized liver, and for other tissue types possessing an expressed solid structure.

Experimental observations [22, 23] also show that B/A increases nearly linearly with solute concentration, but, for a fixed concentration, is relatively insensitive to the molecular weight of the solute molecules.

Also the water fraction of tissue seems to be related to the state of the tissue. Cancerous tissue normally shows a higher water fraction than normal tissue. The water fraction goes from 0.76 in normal liver tissue to 0.90 for multiple myeloma [33]. In general water in tissue may be found as bound water and as free water in equilibrium with one another and expressed by:

$$(H_2O)_n \rightleftharpoons n\, H_2O \qquad (12)$$

where $(H_2O)_n$ is referred to as bound water while H_2O is generally referred to as free water. An increase in the bound state means that, on average, molecules have a greater degree of association with the neighboring molecules which means that they are held more strongly together. This stronger binding also makes a larger ultrasonic pressure necessary in order to stretch the intermolecular bonds into their nonlinear region, which macroscopically is being felt as decreased nonlinearity of the water according to expression (3). This suggests that the magnitude of B/A in water-like media may be related to the relative amounts of bound and free water.

The equilibrium between these two water states, for instance expressed through the ratio of bound to free water, is closely related to the state and the nature of the tissue as shown by NMR studies [39].Prospective relations between the ratio of bound to free water and the nonlinear parameter B/A have been suggested [40]. It was concluded in [40] that the temperature dependence of B/A of water for instance could be due to the change of the ratio of the bound to the free water with the change in temperature. Whether the estimates of the ratios of bound to the free water determined from B/A measurements can be used for characterization of biological media, like for instance human tissue, is still an open question which has to be studied more closely.

The existence of several possible relationships between B/A of biological media and other physical qualities like intermolecular potentials, macro structure, water emphasize the need for further systematic studies. This will most likely demand an internationally (for instance EC) funded research programme where several qualifies laboratories in various countries share a research programme over some years on advanced modelling of biological media.

In spite of the inhomogeneous character of biological materials like for instance tissues, where scattering phase cancellations, dispersion etc. influence the ultrasonic wave propagation, a reliable experimental procedure leading to reproducible B/A-data should be developed to prove, *in vitro*, that B/A may be used for characterization of biological

media. If the uncertainties found in relation to the experimental data could be reduced for the thermodynamic or for the finite-amplitude method, - or maybe for both - a reply to the question about the applicability of B/A for characterization of biological media may be found.

The prospective development of a clinically applicable *in vivo* method - being able to give a two-dimensional picture of the real B/A variation in tissues of various types using non-invasive methods - will form the final step towards a general application of B/A for characterization of biological media on a par with the use of other ultrasonic qualities of these media.

Research work in the three fields mentioned above is at present going on in several laboratories around the world, but there exists a still increasing need for a world-wide collaboration on solving the three closely related problems. This need has been emphasized by creation of evidence for ultrasonic finite-amplitude distortion effects in muscles using standard medical ultrasonic equipment [4]. It is the hope that in the near future not only the vital relations between B/A and the physics, the structure and the state

of biological media may be found, but also that a development of a reliable, reproducible and representative clinical method for "*in vivo*" determination of B/A may take place.

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