The generalized finite amplitude insert-substitution method for B/A measurement of tissues and liquids

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B/A holds promise as a biomarker for tissue characterization. Moreover, measuring B/A enables identifying isomer types in biological liquids, potentially aiding diagnosis of some diseases. The standard finite amplitude insert-substitution method of measuring B/A has several limitations. It assumes a linear dependency of the attenuation coefficient on frequency, limiting it to tissue applications, and necessitates sample positioning close to the receiver. Here we propose the generalized finite amplitude insert-substitution method, which allows for B/A measurement of tissues and liquids, and gives freedom in sample positioning. In this work, we present the derived formula and validate the experimental procedure. For this purpose, B/A measurements of corn oil, porcine fat and porcine liver were conducted. For each substance, several conditions were tested, including various distances between the source and the receiver, various sample positions and amplitudes of the transmitted pulses. A better agreement with literature data was found for lower source pressure amplitudes and distances within the near field of the source transducer. In these conditions, the measurement error was confined to 8% and 25% of the literature values of corn oil and the considered tissues, respectively. No clear indication of the influence of sample position on the measurement accuracy was found.
1. INTRODUCTION

B/A has potential to provide insight for diagnostic tissue characterization.\textsuperscript{1-3} Despite numerous works studying B/A’s utility for medical applications, multiple pathological conditions have not yet been investigated. For example, we were able to identify only two studies, measuring B/A of malignant tissues in comparison to healthy tissues.\textsuperscript{4,5} As for B/A of liquid biological samples, it may aid detection of early onset diabetes\textsuperscript{6} and porphyria type\textsuperscript{7} through identification of isomer type in human biological liquids. Therefore, development of accurate B/A measurement procedures is of clinical relevance. To facilitate this, we present the generalized finite amplitude insert-substitution method (GFAIS). Unlike the original finite amplitude insert-substitution method (FAIS), it allows measurement of samples with an arbitrary frequency dependence and gives great freedom of sample positioning between the source and receiver.

The FAIS\textsuperscript{8,9} method is one of the most common methods utilized for B/A measurement, since it only requires an acoustic source, a receiver and a cuvette to contain the investigated sample. It necessitates little sample volume and eliminates the need for a calibration procedure that converts received voltage signals to absolute pressure values. The method is based on the 2nd harmonic measurement in two configurations: (1) when the source and the receiver are submerged in a reference medium with a known B/A and (2) when the studied medium occupies a fraction of the acoustic path, as in Fig. 1. The original method developed for the measurement of B/A of lossy media\textsuperscript{8} assumes that the investigated medium has a nearly linear frequency dependence of the attenuation coefficient. For this reason, it has been mainly utilized for B/A measurement of biological tissues. Since B/A reflects liquid content and molecular structure,\textsuperscript{10,11} it may also be of use when analyzing biological liquid samples that have a nearly quadratic frequency dependence of the attenuation coefficient. This motivates the development of a practical method that allows measuring B/A of liquids as well as tissues. Besides this, the original FAIS method proposes to set the sample close to the receiver, neglecting 2nd harmonic generation on the last part of the acoustic path. This imposes constraints on the cuvette geometry.

In this study, we derive a formula that takes into consideration 2nd harmonic generation on all parts of the acoustic path and makes no assumptions about the frequency dependence of the investigated specimen. The formula was validated when measuring B/A of corn oil, a slice of porcine liver and a slice of porcine fat. For this purpose, several conditions were tested, including various distances between the source and the receiver, various sample positions and amplitudes of the transmitted pulses. Such a broad spectrum of tested conditions allowed us to test the validity of the assumptions made when deriving the equation for B/A and to indentify the most favorable experimental conditions for an accurate B/A derivation with the proposed equation.

2. MATERIALS AND METHODS

A. THEORETICAL BACKGROUND

The finite amplitude insert-substitution method exploits cumulative 2nd harmonic generation.\textsuperscript{8,9} When an acoustic source emits a monochromatic wave at a frequency \(f\), as this wave propagates, a signal at the 2nd harmonic frequency \(2f\) is accumulated. Its amplitude \(P_2(z)\) at a distance \(z\) from the source is proportional to the parameter of nonlinearity of the propagation medium, expressed by the lossy Fubini solution\textsuperscript{12} for a plane wave propagating in lossy media

\[
P_2(z) = \frac{(2 + B/A)\pi f}{2\rho c^3} P_1^2(0) e^{-\alpha_2 z} - e^{-2\alpha_1 z},
\]

where \(B/A\) is the parameter of nonlinearity, \(\rho\) is the equilibrium density, \(c\) the small-signal speed of sound, \(P_1(0)\) the pressure amplitude at the source, \(\alpha_1\) and \(\alpha_2\) the attenuation coefficients at the fundamental \(f\) and

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2nd harmonic $2f$ frequencies, respectively. This equation was derived assuming 3rd harmonic generation negligible and, therefore, is not suited for high source pressures. If the 2nd harmonic is measured in water, whose $B/A$ is well-known, and a second measurement is conducted when a sample with an unknown $B/A$ is inserted in the path between the source and receiver (Fig. 1), one can determine the $B/A$ of the studied medium from the ratio of the 2nd harmonics in these two configurations. Assuming that the 2nd harmonic contributions generated in various media add up linearly and assuming water to be lossless, one can derive the following formula:

$$\frac{1 + \frac{1}{2}(\frac{B}{A})_{\text{med}}}{1 + \frac{1}{2}(\frac{B}{A})_{\text{wat}}} = \frac{P_{2\text{med}}}{P_{2\text{wat}}} - \frac{a}{L} \frac{D'' D'}{D'} e^{-\alpha_{2\text{med}} d} e^{-2\alpha_{1\text{med}} d} \frac{(\rho c^3)_{\text{med}}}{(\rho c^3)_{\text{wat}}} \frac{2\alpha_{1\text{med}} - \alpha_{2\text{med}}}{L} D''^2 D',$$

(2)

where the subscripts “med” and “wat” refer to the studied medium and water, respectively. Here $D' = 2(\rho c)_{\text{wat}}/[(\rho c)_{\text{med}} + (\rho c)_{\text{wat}}]$ and $D'' = 2(\rho c)_{\text{med}}/[(\rho c)_{\text{med}} + (\rho c)_{\text{wat}}]$ are the pressure transmission coefficients from water to the sample and from the sample to water at normal incidence of the transmitted plane wave. $L$ is the distance between the source and the receiver, $a$ the distance between the source and the sample, $d$ the sample thickness and $b$ the distance from the sample to the receiver. As one can notice from Eq. 2, knowledge of the transmitted pressure $P_1^2(0)$ is not necessary. Moreover, for a linear receiving system, the ratio $P_{2\text{med}}/P_{2\text{wat}}$ is equal to the ratio of the received voltages $V_{2\text{med}}/V_{2\text{wat}}$.

B. EXPERIMENTAL PROCEDURE

The setup consisted of an acoustic source, a plane piston transducer 2.5 cm in diameter (C304-SU, 251 Olympus NDT Inc., Waltham, MA, USA), a receiver, a plane piston transducer 1.3 cm in diameter (V309, Panametrics-NDT, 251 Olympus NDT Inc., Waltham, MA, USA), and a cuvette that were all fixed on a rail system, ensuring their alignment. The cuvette was 3D printed (Fig. 2a). Its sides contained openings for rings that, when inserted, allowed to fix acoustically transparent 3-IN-1 FOLIE (Albert Hein B.V., Zaandam, The Netherlands) food film in place. The acoustically transparent film provided smooth surfaces of the investigated sample, perpendicular to the beam propagation direction.

The US acquisition was controlled via dedicated software written in Labview (National Instruments Corp., Austin, TX, USA) and implemented in a desktop. The driving signals were generated by a 33220A arbitrary wave generator (Agilent Technologies, Santa Clara, USA), further transmitted to a 50-dB 2100L

![Figure 1: Schematic of the setup, submerged in water, utilized to measure $\alpha_1$, $\alpha_2$ and $B/A$. The reference measurement is conducted without the (green) sample between the source and the receiver.](image-url)
RF Power amplifier (Acquitek, Massy, France). The amplified signals were transmitted to the source transducer. In all cases, these were sinusoidal 20-cycle tone bursts with a rectangular window. This pulse length provided a sufficiently narrow bandwidth of the transmitted signals and, therefore, allowed preventing overlap between the harmonics in the spectrum of the received signal. The silence period between the pulses was always set to 250 microseconds, chosen to prevent interference of any possible reflections. A total of 92-95 pulses was transmitted at every acquisition. The received signals were displayed on a TDS2014 oscilloscope (Tektronix U.K. Limited, Bracknell, UK) and sampled throughout an NI-5122 (National Instruments Corp.) acquisition board which was connected back to the desktop. The signals were recorded at a sampling frequency of 25 MHz and saved for off-line analysis.

To estimate $B/A$, the following parameters are required: distances $a$, $b$, $d$, densities $\rho_{\text{med}}$ and $\rho_{\text{wat}}$, speed of sound $c_{\text{med}}$ and $c_{\text{wat}}$, the attenuation coefficients $\alpha_{\text{med}}$ and $\alpha_{\text{med}}$, and 2nd harmonic amplitudes $V_{\text{med}}$, $V_{\text{wat}}$ (see Eq. 2). The distances $a$ and $b$ were measured with a caliper, while $d$ was always taken as 1 cm, equal to the fixed cuvette length. The densities and speed of sound were taken from the literature (Table 1). The rest of the parameters were measured with three through-transmission measurements, utilizing the described setup. The attenuation coefficients $\alpha_{\text{med}}$ and $\alpha_{\text{med}}$ were measured when transmitting a low-amplitude pulse of 0.01 V to the source at its center frequency (2.25 MHz) and its potential 2nd harmonic frequency (4.5 MHz), respectively. Typically, accurate attenuation measurements require low amplitude pulses to prevent energy loss at the fundamental due to higher harmonic generation. $V_{\text{med}}$, $V_{\text{wat}}$ were measured when a high-amplitude pulse was transmitted at 2.25 MHz, resulting in 2nd harmonic generation. Since the validity of Eq. 1 is dependant on the source pressure amplitude, several high-amplitude acquisitions were performed, transmitting signals of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 V to the source transducer. A $B/A$ estimate was computed for each of these amplitudes.

For the measurement, the setup was submerged in a tank filled with degassed water at room temperature. Since the advantage of the FAIS methods is that no source calibration is required, no such procedure was performed. The distance between the source and the receiver was set to one of the four tested distances: 4, 5, 6 or 7 cm. These distances were chosen to ensure sufficient space between the source and the receiver and, therefore, a convenient positioning of the cuvette inbetween. At the same time, the plane wave approximation is valid in the proximity of the source, so further distances were not studied. A first acquisition without the sample registered the signals received from the transmission of 2 low-amplitude pulses and 7 high-amplitude pulses, as described above. Then the investigated sample was fixed at one of the three positions between the source and the receiver (Fig. 1): close to the source (with its front face 0.5 cm away from the source), in the middle between the source and the receiver, and close to the receiver (with its rear face 0.5 cm away from the receiver). Corn oil could be poured directly in the cuvette when the thin food film was still fixed on one side only. Since it was difficult to cut tissue samples with smooth side surfaces, tissue was frozen to increase its stiffness prior to the slicing procedure, and a dedicated device (Fig. 2b) was utilized to cut it in slices of homogeneous thickness with parallel sides. As the cuvette was 1 cm thick, the

<table>
<thead>
<tr>
<th>Medium</th>
<th>$\alpha_{\text{med}}$, dB/cm</th>
<th>$\alpha_{\text{med}}$, dB/cm</th>
<th>$\rho_{\text{med}}$, kg/m$^3$</th>
<th>$c_{\text{med}}$, m/s</th>
<th>$B/A$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>0.3</td>
<td>1.2</td>
<td>920</td>
<td>1466</td>
<td>10.5</td>
</tr>
<tr>
<td>Porcine liver</td>
<td>1.2</td>
<td>2.1</td>
<td>1050</td>
<td>1611</td>
<td>6.3</td>
</tr>
<tr>
<td>Porcine fat</td>
<td>1.0</td>
<td>2.2</td>
<td>970</td>
<td>1460</td>
<td>10.8</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>0</td>
<td>1000</td>
<td>1509</td>
<td>5.2</td>
</tr>
</tbody>
</table>

The values of acoustic parameters were taken from Ref.14 for corn oil, from Ref.8 for tissues, and Ref.15 for water. Water is assumed lossless.
Figure 2: (a) The cuvette utilized for all samples. The outer rings are inserted in the cuvette opening to hold the food film tight. (b) The device utilized to cut tissue into slices with smooth parallel sides. The needles hold the tissue phantom in place, while the blade is inserted in steel openings perpendicular to the bottom of the device.

prepared tissue slices were 1 cm thick. Further, they were degassed in a degassing chamber with saline for 40 minutes. After degassing, they were fixed in the cuvette. This procedure was performed under water to avoid trapping air bubbles. The thin film provided further correction for possible thickness inhomogeneities during the slicing procedure. Once the investigated sample was positioned, the acquisition was repeated as for the pure water path. The sample was then moved to the next position between the source and the receiver and the acquisition was repeated again.

C. DATA ANALYSIS

Data analysis was performed in MATLAB® (The MathWorks, Inc., Natick, MA, USA). The beginning of every pulse in each acquisition was identified, and the central segment of the pulse with a stable amplitude was selected. For attenuation measurements, the central segment of every pulse was directly processed by the Fast Fourier Transform. The average Fourier spectrum among all pulses in an acquisition was calculated and the fundamental harmonic component was extracted from it. With the amplitude of the fundamental component for the pure water path $V_{\text{wat},2f}$ and for the path with the sample $V_{\text{med},2f}$, and the pressure transmission coefficient $T$ accounting for two transmission boundaries (water-sample, sample-water), the attenuation coefficients were calculated as

$$\alpha_{f,2f} = \frac{20}{d} \log_{10}(T \frac{V_{\text{wat},2f}}{V_{\text{med},2f}}),$$

for frequencies $f=2.25$ MHz and $2f=4.5$ MHz, respectively. As for the high-amplitude pulses transmitted to record 2nd harmonics $V_{\text{med},2}$, $V_{\text{wat}}$ (Eq. 2), a Hanning window was applied to the central part of the pulses and an average Fourier spectrum of the resulting signals was calculated. The Hanning window minimized spectral leakage from the strong fundamental component to the weaker 2nd harmonic.\textsuperscript{18} The 2nd harmonic component of the average Fourier spectrum was extracted to compute $V_{\text{med},2}/V_{\text{wat},2}$ for every tested amplitude.
3. RESULTS

The measured $\alpha_{1\text{med}}$, $\alpha_{2\text{med}}$ and $V_{2\text{med}}/V_{2\text{wat}}$ for corn oil are presented in Fig. 3 for various distances between the source and the receiver $L$, as well as sample positions. Colors encode different transmitted amplitudes of the high-pressure pulses inducing 2nd harmonic generation. The measured parameters demonstrated in Fig. 3 were inserted in Eq. 1 to extract $B/A$ for the tested experimental conditions. The resulting $B/A$ estimation is demonstrated in Fig. 4.

From Fig. 3, one can see that the measured attenuation coefficients are slightly greater than the literature values for corn oil (Table 1), especially at the 2nd harmonic frequency. Despite this, there is good agreement between the measured $B/A$ values and the literature value of 10.5. The standard deviation at the largest distance between the source and the receiver is appreciably greater, compared to shorter distances. We hypothesize that the plane wave approximation is no longer valid at this distance. If we assume that the effective radius of our source is equal to its geometrical radius $r$, the near field where the plane wave approximation is valid is up to about $0.3 \cdot r^2 / c = 7 \text{ cm}$ away from the source (Fig. 9 in,\textsuperscript{17} page 354), making the distance of 7 cm the threshold value, less appropriate for an accurate measurement. Despite this, the error is within 6% of the literature value for the lowest transmitted amplitude of 0.1 V at all source-receiver separation distances.

No consistent influence of the amplitude of the high-amplitude pulses on $B/A$ accuracy was noticed (Table 2). However, when averaged over all experimental configurations, the lowest amplitude demonstrated the best agreement with the literature values. In fact, when visually inspecting the spectra of the acquired signals in water, already at the shortest distance of 4 cm and at a transmitting amplitude of 0.3 V, a 3rd harmonic component arises, constituting 25% of the 2nd harmonic amplitude. This brings us to the conclusion that sonicating amplitudes of 0.3 V and greater should already lead to an overestimation of $B/A$. Looking at Table 2, we see that the amplitudes of 0.3-0.4 V yield larger $B/A$ values for all studied samples, compared to lower amplitudes. Interestingly, the values decrease for amplitudes higher than 0.4 V, which may indicate a nonlinear conversion factor in the receiver, i.e. in the electromechanical conversion between...
pressure and voltage.

Sample positions close to the source yield greater values than those close to the receiver. However, for the lowest amplitude, the estimated \( B/A \) values are nearly equivalent.

The measured \( \alpha_{1\text{med}}, \alpha_{2\text{med}} \) and \( V_{2\text{med}}/V_{2\text{ref}} \) for porcine liver and porcine fat are shown on Fig. 5 and 6, respectively. No measurement was conducted for \( L = 7 \) cm, as it was expected to yield even more variation in \( B/A \) of inherently inhomogeneous tissues. The amplitude of 0.1 V is not presented on these graphs, since the signal-to-noise ratio of the 2nd harmonic signal was too low for an accurate \( B/A \) estimation.

From these graphs, one can notice that the measured attenuation coefficients are overestimated, com-

**Figure 4:** The measured \( B/A \) for corn oil at various distances between the source and the receiver \( L \), at various amplitudes and different sample positions: +(close to the source), *(close to the receiver), ◦(in the middle). The thick gray line indicates the literature value of 10.5.

**Table 2:** Mean \( B/A \) for different voltages transmitted to the source.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Volts</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>0.4</th>
<th>0.5</th>
<th>0.6</th>
<th>0.7</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td></td>
<td>10.7</td>
<td>11.0</td>
<td>11.1</td>
<td>11.3</td>
<td>10.8</td>
<td>10.8</td>
<td>10.9</td>
<td>10.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Porcine liver</td>
<td>-</td>
<td>6.4</td>
<td>6.7</td>
<td>6.8</td>
<td>5.3</td>
<td>5.9</td>
<td>5.9</td>
<td>6.3&lt;sup&gt;b&lt;/sup&gt;, 6.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Porcine fat</td>
<td>-</td>
<td>11.9</td>
<td>12.6</td>
<td>13.3</td>
<td>13.3</td>
<td>13.7</td>
<td>13.2</td>
<td>9.1&lt;sup&gt;b&lt;/sup&gt;, 10.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> see Ref.<sup>19</sup>
<sup>b</sup> Ref.<sup>20</sup>
<sup>c</sup> Ref.<sup>1</sup>

The presented \( B/A \) values were averaged among all measurements conducted at different source-receiver separation distances and sample positions. The voltage is stated in brackets, in volts.
Figure 5: The attenuation coefficients at the fundamental $\alpha_{1\text{med}}$ and 2nd harmonic $\alpha_{2\text{med}}$ and the ratio of 2nd harmonic amplitudes $V_{2\text{med}}/V_{2\text{ref}}$ for porcine fat. The measurements were conducted at several distances between the source and the receiver $L$ and various sample positions: +(close to the source), *(close to the receiver), o(in the middle). Colors for $V_{2\text{med}}/V_{2\text{ref}}$ measurment encode different amplitudes, transmitted to the source.

pared to the literature values, most prominently for fat. For both tissue samples, overestimation is greater for 4.5 MHz, compared to 2.25 MHz. We hypothesize that this occurs due to greater phase-cancellation effects at higher frequencies. Phase-cancellation is much more prominent for tissues, compared to liquids, as tissues have intrinsic inhomogeneities in the speed of sound, leading to different arrival times for different beam portions. There is great variation in the measured attenuation coefficients of porcine liver among different source-receiver separation distances.

Despite the variation and inaccuracies in the estimation of the attenuation coefficients, we again see that the estimated $B/A$ is close to the literature values for distances of 4 cm and 5 cm between the source and receiver and amplitudes up to 0.3 V (Fig. 7, Table 2). In case of fat, the error in the $B/A$ measurement grows, overestimating $B/A$, as the sample is positioned further away from the receiver. This effect is most prominent for amplitudes in the range 0.5-0.7 V. As for porcine liver, we also see a tendency of sample positions closer to the source to overestimate $B/A$. However, a proper analysis of the influence of the sample positioning on the measurement accuracy is hindered by the variability in literature data for tissues (Table 2). Besides this, the cuvette utilized in the measurement was not optimal for measurements with tissue. A cuvette with two separate sides, allowing to gently squeeze the tissue and measure its thickness more accurately would enable more accurate analysis.

4. CONCLUSIONS

The current experiments demonstrated that the derived formula and the utilized setup are capable of measuring $B/A$ of media with different frequency dependences of the attenuation coefficient. For all media, lower amplitudes and shorter distances between the source and the receiver favored better $B/A$ agreement with literature values. Amplitudes of 0.3 V and lower resulted in a maximum error of 25% and 8% for tissues and corn oil, respectively, for all configurations at shorter distances of 4 and 5 cm. We conclude that for tissues the utilized source transducer should not be driven at an amplitude higher than 0.3 V for a $B/A$ estimation within 25% accuracy. At higher amplitudes, we observed nonnegligible 3rd harmonic generation and, possibly, nonlinearities in the receiving system.
Figure 6: The attenuation coefficients at the fundamental $\alpha_{1\text{med}}$ and 2nd harmonic $\alpha_{2\text{med}}$ and the ratio of 2nd harmonic amplitudes $V_{2\text{med}}/V_{2\text{ref}}$ for porcine liver. The measurements were conducted at several distances between the source and the receiver $L$ and various sample positions: + (close to the source), *(close to the receiver), ◦ (in the middle). Colors for $V_{2\text{med}}/V_{2\text{ref}}$ measurement encode different amplitudes, transmitted to the source.

Figure 7: Estimated $B/A$ for (a) Porcine liver; (b) Porcine fat. The symbols encode sample positions: + (close to the source), *(close to the receiver), ◦ (in the middle).

The data clearly indicates more accurate $B/A$ estimations for the liquid corn oil, compared to tissues. This is typical for FAM measurements, since tissue has an inherently heterogeneous structure, leading to greater phase-cancellation effects on the receiver. These effects are enhanced for large receivers and can contribute to great overestimation of the attenuation coefficients in tissues. Despite this, the predicted $B/A$ values are in the range of expected values. Therefore, we conclude that the FAIS and its modification require the measurement of attenuation as a whole, including absorption, diffraction, dispersion and phase...
cancellation effects.

Even though overall there is a tendency for $B/A$ to be overestimated at sample positions further away from the receiver, this effect is very limited for the lowest amplitudes, within the near field. It is difficult to draw conclusions on the generalizability of this effect, which may depend on the specific design of the cuvette and is difficult to compare to the literature due to the variability of the published estimates (Table 1). Yet, based on this work, we did not identify a markable influence of the sample position on the accuracy of $B/A$ estimation. However, extensive analysis, also involving different cuvette designs and a smaller receiver, are required to derive more general conclusions on this aspect. The experiment may also benefit from a phased array source, since it may provide a closer resemblance of the field to that of a plane wave, also at further (axial) distances from the source.

This work described an improved $B/A$ measurement method and demonstrated a validation process of the proposed approach and measurement procedure. We identified the experimental conditions that do not violate the assumptions made when deriving the proposed equation and, therefore, yield accurate $B/A$ estimates. A smaller receiver is hypothesized to reduce phase-cancellation effects and yield more accurate $B/A$ estimates.

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