Assessment of ultrasonic properties of an agarose phantom at the frequency range 2.25 MHz to 10 MHz

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Abstract. Agarose-based phantoms are widely used to evaluate ultrasonic equipment used for diagnosis. Many studies report phantom’s ultrasonic characteristics as a function of frequency, mainly sound velocity and attenuation, but a metrological approach is not widely spread. The current paper reports attenuation coefficient (Att-c) and speed of sound (SoS) in an agarose-based phantom as a function of the diagnostically used frequency, ranging from 2.25 MHz to 10 MHz, at 21 °C. The pulse-echo method was applied to measure the ultrasonic properties. The measurements were performed under repeatability conditions (n = 4) and the expanded uncertainties of the measurements were estimated thereafter. It was observed that there was no statistical difference among the values of SoS (average of 1,540.3 m s\(^{-1}\)) for all frequencies, in which the maximum \(E_n = 0.98\). The SoS result with the highest uncertainty was of 1,538.7 m s\(^{-1}\) ± 3.8 m s\(^{-1}\) (\(k = 2\); coverage probability \(p = 0.95\)). Att-c increases as a power function of frequency (Att-c = \(f^{1.09}\); \(a_0 = 0.573\); \(R^2 = 0.9995\). A survey of the literature was made and it was found that Att-c values obtained in this research are consistent with the previously reported. Based on the SoS, this study draws attention to the importance of performing a statistical test to assess the consistency of the results.

1. Introduction
Ultrasonic phantoms or Tissue Mimicking Materials (TMMs) are intended to mimic the ultrasonic (US) properties of biological tissues, such as group speed of sound, attenuation coefficient, and backscatter coefficients. These phantoms play an important role in the evaluation of performance, safety, and calibration of diagnostic and ultrasonic therapy equipment, as well as in studies of the biological effects caused by ultrasound [1][2].

They shall mimic the ultrasonic properties of biological tissues in the temperatures and frequencies ranges used in both therapeutic and diagnostic applications. It is necessary as the frequency may vary depending on the application or the patient characteristics. In the diagnostic applications, for instance, the choice of transducer frequency may vary according to the beam penetration and axial resolution required. Abdominal transducers with frequencies between 3 MHz to 5 MHz allow sufficient penetration and provide adequate resolution for most patients. Lower-frequency transducers (about 2 MHz) may be needed to provide adequate penetration for abdominal imaging in obese patients. In the
early pregnancy, an abdominal transducer of 5 MHz or vaginal transducers in the range 5 MHz to 10 MHz may provide superior resolution, while still allowing adequate penetration [1][3].

The standard IEC 60601-2-37 [4] describes the particular basic safety and essential performance requirements for ultrasonic diagnostic equipment. The reference values of the acoustic and thermal properties of an agar-based soft TMM are also disclosed [4]. This standard recommends a speed of sound of 1,540 m s\(^{-1}\) and attenuation coefficient of 0.5 dB cm\(^{-1}\) MHz\(^{-1}\) estimated at a single frequency of 3 MHz [4].

There are many studies about the effects of frequency on ultrasonic properties of phantoms [5]. According to the literature, considering non-dispersive media, the speed of sound does not vary as a function of frequency, whereas the absorption does, impacting on the attenuation coefficient values. Burlew et al. [6] evaluated the influence of powdered graphite concentration on attenuation coefficient values in five samples of agar-based soft TMM. They also studied the behavior of the attenuation coefficient over the frequency range of 1 MHz to 7 MHz, all samples at 22 °C. The curves demonstrated a nearly proportional relation between the attenuation and the frequency. Wear et al. [7] produced phantoms described in [6] and carried out an interlaboratory comparison of ultrasonic backscatter, attenuation coefficient, and speed of sound measurements. The attenuation coefficient was measured at a frequency range from 2.5 MHz to 8 MHz at 22 °C. Browne et al. [8] investigated the ultrasonic properties of different TMMs at the frequency range from 2.25 MHz to 15 MHz at room temperature. The agar-based phantom had a linear response of attenuation to frequency. Brewin et al. [9] characterized the ultrasonic properties of agar-based TMMs at ultrasound frequencies centered on 20 MHz. The speed of sound was found to be independent of frequency in this range.

Based on the literature, it is noted that few works report the uncertainty of measurements of ultrasonic properties. Others reported only standard deviation or just the mean value. According to the Guide to the Expression of Uncertainty in Measurement (GUM) [10], the experimental standard deviation of the arithmetic means or average of a series of observations is a measure of the uncertainty of the mean due to random effects. However, it is understood that the result of the measurement is the best estimate of the value of the measurand and that all components of uncertainty, including those arising from systematic effects, such as components associated with corrections and reference standards, contribute to the dispersion [10]. It is important to highlight that taking account of all components of measurement uncertainty may contribute significantly to provide reliability to the performance testing of ultrasound equipment [11].

Based on the above, this paper aims to study the influence of all quantities associated with the process of measuring ultrasonic properties of an agarose-based soft TMM as a function of the diagnostically-used frequency range from 2.25 MHz to 10 MHz at 21 °C.

2. Materials and methods

2.1. Phantom

The standardized agarose-based phantom was prepared from the components disclosed in [4] and according to the standard operating procedure described in [12]. The ultrasonic propagation velocity and attenuation coefficient of a cylindrical phantom sample (Ø 55 mm × 28 mm) were investigated. According to [4], the soft TMM shall have the SoS and the attenuation coefficient of 1,540 m s\(^{-1}\) and 0.5 dB cm\(^{-1}\) MHz\(^{-1}\), respectively.

2.2. Measurement of the speed of sound and attenuation coefficient

It was employed the pulse-echo method, in which the transducer acts as a transmitter and receiver, inside a water tank, and it is driven by a wave generator (See figure 1). More details regarding the measurement system can be found somewhere else [1]. The measurements were performed at 21 °C. To study the frequency-dependent ultrasonic properties, five transducers (Olympus, USA) were used with center frequencies of 2.25 MHz (model A305S), 3.5 MHz (model A380S), 5 MHz (model A307S), 7.5 MHz (model A320S), and 10 MHz (model A311S). For the maximization of RF signals, the transducer was
aligned with a reflecting target inside the water bath. The temperature of the water was measured using a calibrated thermometer.

**Figure 1 – Measurement system. A: water bath; B: thermometer; C: agarose-based phantom; D: alignment system of the transducer; E: oscilloscope; F: signal generator.**

2.3. Measurement uncertainty of the ultrasonic properties
To estimate the uncertainty of the measured ultrasonic properties, it was used the Guide to the Expression of Uncertainty in Measurement (GUM) [10]. The Type A evaluation of uncertainty was carried out by the statistical analysis of four observations (no = 4) of each ultrasonic property under repeatability conditions. Other input quantities were considered on the Type B evaluation of uncertainty. The sources of uncertainty considered for both ultrasonic properties are better described in [1]. Besides that, to include the influence of the power fit for all properties measurements, the mean squared error (MSE) was also considered on uncertainty calculation for both US properties.

2.4. Normalized error test
The normalized error ($E_n$) (equation 1) was calculated to assess if there was any statistically significant difference between the SoS results.

$$E_n = \frac{|X_{f1} - X_{f2}|}{\sqrt{U_{f1}^2 + U_{f2}^2}}$$

(1),

in which $X_{f1}$ and $X_{f2}$ are the mean values of speed of sound measured at different frequencies, whilst $U_{f1}$ and $U_{f2}$ are the respective expanded uncertainties. If the maximum normalized error is less than 1.0 ($E_n \leq 1$), it can be inferred that the measurement results are not statistically different.
3. Results

3.1. Frequency dependence of the speed of sound
Table 1 and Figure 2 show the results of the four replicates of the speed of sound measurements for each frequency used.

Table 1 – Speed of sound as a function of the frequency

| Temp [°C] | Freq [MHz] | Ultrasonic Velocity [m s⁻¹] | Type A Uncertainty [m s⁻¹] | Type B Uncertainty [m s⁻¹] | Fit Uncertainty [m s⁻¹] | Coverage Factor | Expanded Uncertainty [m s⁻¹] |
|-----------|------------|-----------------------------|---------------------------|---------------------------|------------------------|----------------|-----------------------------|
| 21.2      | 2.25       | 1,538.7                     | 0.34                      | 1.69                      |                        | 2              | 3.8                         |
| 21.0      | 3.5        | 1,538.7                     | 0.31                      | 1.03                      |                        | 2              | 2.7                         |
| 21.0      | 5          | 1,540.3                     | 0.25                      | 0.96                      | 0.74                   | 2              | 2.5                         |
| 21.0      | 7.5        | 1,542.3                     | 0.26                      | 0.92                      |                        | 2              | 2.5                         |
| 21.2      | 10         | 1,541.3                     | 0.25                      | 0.96                      |                        | 2              | 2.5                         |

Figure 2 – Speed of sound as a function of the frequency in the range from 2.25 MHz to 10 MHz.

3.2. Frequency dependence of the attenuation coefficient
Table 2 and Figure 3 show the results of the four replicates of the attenuation coefficient measurements for each frequency employed. Figure 3 shows a nearly linear response of attenuation to frequency.

Table 2 – Attenuation coefficient as a function of the frequency

| Temp [°C] | Freq [MHz] | Attenuation Coefficient [dB cm⁻¹] | Type A Uncertainty [dB cm⁻¹] | Type B Uncertainty [dB cm⁻¹] | Fit Uncertainty [dB cm⁻¹] | Coverage Factor | Expanded Uncertainty [dB cm⁻¹] |
|-----------|------------|---------------------------------|-----------------------------|-----------------------------|--------------------------|----------------|-----------------------------|
| 21.2      | 2.25       | 1.39                            | 0.0038                      | 0.0036                      |                         | 2              | 0.14                        |
| 21        | 3.5        | 2.28                            | 0.012                       | 0.0034                      |                         | 2              | 0.14                        |
| 21        | 5          | 3.27                            | 0.023                       | 0.0036                      | 0.068                   | 2              | 0.15                        |
| 21        | 7.5        | 5.07                            | 0.025                       | 0.0040                      |                         | 2              | 0.15                        |
| 21.2      | 10         | 7.18                            | 0.0057                      | 0.0051                      |                         | 2              | 0.14                        |
4. Discussions and Conclusion

This paper presents a metrological assessment of the frequency dependence of the speed of sound and the attenuation coefficient of a soft agar-based phantom at a diagnostically-used frequency ranging from 2.25 MHz to 10 MHz.

Diagnostic ultrasound periodical quality control measurements are recommended to ensure the performance, accuracy, and safety of medical ultrasonic imaging equipment [13]. For US image assessment, ultrasonic phantoms shall mimic ultrasonic properties of biological tissue. The ultrasonic frequency is related to the resolution of the US image and the rate of attenuation of the beam in its propagation through the tissue [13].

Ultrasonic propagation velocity of these phantoms should be tracked for different frequencies because of the calibration velocity of the ultrasound scanner, which is 1,540 m s\(^{-1}\) [8]. According to [13], the TMM’s speed of sound shall be constant for the frequency range used in diagnostic applications. If the TMM SoS varies for different frequencies, errors in distance measurements can occur, affecting the lateral resolution assessment.

According to the results (see figure 2 and Table 1), for the frequency range studied at 21\(^{\circ}\)C, it can be inferred that there is no statistical difference among the speed of sound (average of 1,540.3 m s\(^{-1}\)), in which the maximum normalized error was less than 1.0 (\(E_n = 0.98\)). In addition, the SoS result with the highest expanded uncertainty was of 1,538.7 m s\(^{-1}\) ± 3.8 m s\(^{-1}\) (\(k = 2\); coverage probability \(p = 0.95\)).

As can be seen in Figure 3, the TMM attenuation coefficient was found to increase over the higher frequency range and is consistent with the results reported at the diagnostically used frequency range at room temperature (\(\sim\)21 \(^{\circ}\)C). To compare the present results with those from literature, attenuation coefficients were fit to the power-law \(\alpha(f) = \alpha_0 f^n\), where \(f\) is the frequency, and \(\alpha_0\) [dB cm\(^{-1}\) MHz\(^{-n}\)] and \(n\) are constants obtained by the curve fitting. This approach was chosen here only to compare our results with the literature. However, the authors defend that the correct way is to present attenuation coefficient results for each frequency study, as shown in Table 2. It is worth mentioning that the fractional power of frequency has no physical meaning, but it is just a mathematical approach to fit numerical data with an analytical regression.

In the present paper, the attenuation coefficient fit had \(\alpha_0 = 0.57\) and response of \(f^{1.09}\) (\(R^2 = 0.9995\)). Browne et al. [8] reported responses of attenuation coefficient at 20 \(^{\circ}\)C of \(f^{1.01}\) (\(\alpha_0 = 0.53\)), \(f^{1.09}\) (\(\alpha_0 = 0.72\)) and \(f^{1.3}\) (\(\alpha_0 = 0.48\)) to frequency range of 2.25 MHz to 15 MHz in agar-based, condenses milk gel and zerdine TMMs, respectively. Moreover, the uncertainty of the attenuation coefficient measurement was of 0.03 dB cm\(^{-1}\). They also reported a relatively constant SoS (uncertainty of 1 m s\(^{-1}\)) with increasing frequency for all TMM tested [8]. Although no average value has been reported, the following SoS results at 20 \(^{\circ}\)C were displayed: agar TMM (1,546 m s\(^{-1}\) at 2.25 MHz and
1,547 m s$^{-1}$ at 15 MHz; condensed milk gel TMM (1,545 m s$^{-1}$ at 2.25 MHz and 1,542 m s$^{-1}$ at 15 MHz); and zerdine TMM (1,538 m s$^{-1}$ at 2.25 MHz and 1,536 m s$^{-1}$ at 15 MHz).

In a study by Nam et al. [14], agar-based TMM ultrasonic properties were investigated. The speed of sound was 1,539 m s$^{-1}$ and it was analyzed a power fit to understand the behavior of the frequency-dependence (2.5 MHz to 10 MHz) of the attenuation coefficient. The response of the attenuation coefficient to frequency was $f^{1.11} (\alpha_0 = 0.49)$.

Regarding an interlaboratory comparison of TMMs ultrasonic properties measurements (SoS, attenuation coefficient, and backscatter coefficient) at a frequency range of 2.5 MHz to 9 MHz at 22 °C, Wear at al. [7] affirmed that attenuation coefficient measurement is perhaps the most reliable, with little variation between sites, but no uncertainties were reported. In complement, yet according to [7], higher non-linear responses of the attenuation to the diagnostically-used frequency range may cause errors in the axial resolution measurements and penetration depth distortions may be perceived with the frequency increasing.

Brewin et al. [9] measured the ultrasonic properties of agar-based TMMs at the frequency range of 17 MHz to 23 MHz. The SoS mean value reported was of 1,537.1 m s$^{-1}$. They assumed that the SoS remained constant (varying between 1,533 m s$^{-1}$ and 1,539 m s$^{-1}$), with increasing frequency. They also reported that the attenuation coefficient increased with frequency at a rate of 0.48 dB cm$^{-1}$ MHz$^{-1}$ obtained from the linear fit ($R^2 = 0.99$). For both US properties results, the authors only reported standard deviations.

Sun et al. [15] studied the ultrasonic properties of a standard agar-based phantom [4] at ultrasound frequencies in the range of 10 MHz to 47 MHz. A broadband reflection substitution technique was employed using two independent systems (preclinical ultrasound scanner and scanning acoustic microscope) at 21 °C ± 1 °C. The measured US velocities, with respective standard deviations, were 1,547.4 m s$^{-1}$ ± 1.4 m s$^{-1}$ and 1,548.0 m s$^{-1}$ ± 6.1 m s$^{-1}$, respectively. According to the authors, the speed of sound did not vary significantly over the frequency range.

Rajagopal et al. [16] characterized the SoS and attenuation coefficient of a standard agar-based TMM [4] over the frequency range of 1 MHz to 60 MHz. They identified sources of uncertainty such as sample thickness measurements and the influence of interfacial losses and estimated expanded uncertainties. Non-linear responses of the attenuation to frequency were perceived above 20 MHz [16], achieving a value of 0.93 dB cm$^{-1}$ MHz$^{-1}$ ± 0.04 dB cm$^{-1}$ MHz$^{-1}$ at 60 MHz, determined at a temperature of 21 °C ± 0.5 °C. The authors also estimated the expanded uncertainty of the SoS measurements (1,544 m s$^{-1}$ ± 3.1 m s$^{-1}$) with a coverage factor $k = 2$ and a confidence level of 95 %. They measured the dispersion in SoS measurements and found an increase of 6 m s$^{-1}$ over the frequency range investigated.

In the current paper, to metrologically assess the ultrasonic properties, it was used the Guide to the Expression of Uncertainty in Measurement and the uncertainty model described in [1]. It was considered the Type A and Type B standard uncertainties and different input quantities were taken into account. It is important to highlight that there are a lot of influence quantities that can affect the measurement result of the ultrasonic properties. Therefore, the uncertainty in measurement is not only related to the experimental standard deviation of the arithmetic mean, but also to the experimental setup, to the instruments calibration certificate, and the measurement method employed, for instance.

As previously discussed, the attenuation coefficient results of this work showed a good comparison with the results reported in the literature. Regarding SoS, most studies do not analyze the dispersion of SoS values as a function of frequency. They only assume that the SoS is constant, or approximately constant. Nevertheless, [16] identified that there was dispersion in SoS measurements at the frequency (large) range studied. In the present study, the normalized error was less than 1.0 indicating that the SoS values were not statistically different over the frequency range studied. Since the TMM speed of sound is expected to remain constant as a function of frequency, it is important to perform a statistical analysis to assess the consistency of the TMM SoS values.
Acknowledgments
This work was partially supported by The Brazilian National Council for Scientific and Technological Development, CNPQ [grant 312501/2017-0; grant: 401685/2016-0], and the Pronametro [02/2015].

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