Versatile Ultrasonic Spectrometer for Liquids With Practical Sample Handling by Using Standard Cuvettes

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Abstract—This work describes the development and tests of a new ultrasonic spectrometer for liquids based on the use of commercial cuvettes for sample handling. The spectrometer operates in the frequency range from 20 to 80 MHz and gathers some important characteristics, which are its high thermal stability (better than 0.01 °C), by the use of Peltier cells, and practical sample handling with small volume (≤ 3 ml) samples placed inside cuvettes which can be easily removed from the spectrometer, cleaned/sterilized, or simply discarded. Through-transmission operation is used to measure propagation velocity and attenuation coefficient, and the spectrometer was tested with mixtures of water and NaCl, which have attenuations smaller than that of distilled water, and higher attenuation samples of silicone and castor oil. Backscattering studies of polystyrene particles of 10- and 15-μm diameters were also conducted, showing the versatility of the instrument.

Index Terms—Attenuation, backscattering, cuvettes, propagation velocity, ultrasonic spectrometer, water and NaCl mixtures.

I. INTRODUCTION

ACOUSTIC parameters such as propagation velocity, attenuation coefficient, and acoustic impedance are not only fundamental properties of materials but they can also be used to indirectly obtain other characteristics such as compressibility, elastic constants, concentration, and density [1], [2]. These parameters can be used to evaluate product quality/composition, detect possible adulterations, contaminations, and so on, without the need of dilution and in optically opaque samples [3]–[5].

Ultrasonic spectrometers measure acoustic parameters as a function of frequency and instrument designs vary, depending on the frequency range and measurement principle.

Wideband spectrometers are in general based on resonance methods [6], [7], cover frequency ranges from kilohertz to gigahertz, but require very precise mechanical machining and assembly. Pulsed systems are mainly used in the range of 1–10 MHz [8]–[11], but there are also higher frequency systems that reach 35 MHz [12] and hundreds of megahertz [13]–[15]. Fixed sample path length cells simplify mechanical assembly but require calibration with a known liquid, in general, distilled water, in order to measure the sample path length. Variable path length spectrometers [13], [14] allow absolute measurement of propagation velocity and attenuation coefficient, albeit with increased mechanical complexity.

When developing instruments for liquids characterization, most efforts are directed toward the physical measurement principle, with the objective of attaining high resolution, accuracy, and repeatability. One important aspect, of functioning interest, is related to sample manipulation, especially when dealing with viscous fluids, suspensions, or contaminating samples, such as biological fluids with microorganisms. In these cases, the cleaning conditions or even sterilization requirements of the sample chamber and other elements that are in contact with the liquid (tubes, syringes, or pipette tips) are features of maximum relevance in the laboratory and industrial environments. Very often, measurement devices incorporate sample chambers with difficult access, demanding the disassembly of (part of) the cell for thorough cleaning [8], [16], [17]. For this reason, the use of liquid holders or cuvettes for easier sample handling is advantageous when working with liquids. This is the strategy followed by modern spectrophotometers and fluorimeters, which are widely spread in many laboratories for electromagnetic liquid characterization. Nevertheless, the use of cuvettes may have negative consequences to repeatability and accuracy, because of their size variability and difficulties to achieve an adequate thermal equilibrium.

McClements and Povey [8] used a transducer in direct contact with a cuvette, which was immersed in a water bath for temperature control, operating in a pulse-echo mode in the range of 1–10 MHz. Mather *et al.* [18] also used a cuvette immersed in a water bath, but the transducer was not in direct contact with the cuvette walls, existing a water path between the transducer and cuvette wall. There was no temperature control, and the transducer central frequency was 4 MHz. More recently, Norisuye *et al.* [9] also proposed a similar

system using commercial plastic cuvettes immersed in a water bath. The transducer operated at 10 MHz in the pulse-echo mode, and simultaneous measurements of propagation velocity, attenuation coefficient, and density/acoustic impedance were performed. Elvira *et al.* [19] used a glass bottle instead of a cuvette, and two transducers operating in the through-transmission mode directly attached to the bottle walls. The system had an electronic thermal control and the advantage of using bottle caps, reducing the risk of sample contamination by air. However, the bottle circular geometry hinders proper transducer alignment, diminishing measurement precision.

There are some commercial designs that also employ cuvettes. This is the case of DT-110 from Dispersion Technology Inc., which uses custom-made cuvettes [15]. In addition, there are measurement systems adapted for in-line ultrasound measurements, like SPRn-4115 from Anton Paar, which measure propagation velocity and is easier to clean. However, none of them incorporate thermal control.

Ultrasound backscattering can also be used to characterize liquid samples, by estimating particle size distribution [20] and concentration of particle or bubble suspensions [21], [22]. When using high frequencies, the backscattering signal is often obtained with the transducer in direct contact with the liquid suspension [23], to avoid interferences due to sample chamber walls (reflections and attenuation). Experimental arrangements made for acoustic backscattering describe sample holders of different nature, like stainless steel and latex [24], saran wrap [25], and so on, which are immersed in a water tank for acoustic coupling. Sample holders also become mandatory when small volumes of suspensions have to be studied.

Temperature plays a fundamental role in the measurement of acoustic parameters, and consequently, temperature control is imperative for high accuracy ultrasonic systems. Most thermal control systems described in the literature rely on thermostatic water baths, where the spectrometer or cuvette/sample holder is immersed, or there is a thermostated recirculating liquid around the sample chamber. Although the temperature variations can be as small as 1 mK [9], in the case of water baths, it is necessary to use considerable volumes of water and the corresponding thermal systems are noisy, bulky, and power consuming. The use of Peltier cells allows the elimination of the water bath or recirculating liquids, at the same time, reducing the system volume and noise, and attaining efficient temperature control [19].

The present work reports an ultrasonic spectrometer system based on the use of commercial cuvettes for handling liquid samples. This device combines several positive characteristics: the use of a wide variety of commercial cuvettes (0.5–3 ml volume), that can be easily removed from the spectrometer, cleaned/sterilized, or even discarded; dry thermal control by using Peltier cells, reaching temperature variations of the order of 5 mK; measurement frequency range from 20 to 80 MHz in the pulsed mode. Sample path length is determined by the cuvette, and typical values are 10, 4, or 2 mm. It can be used for sound velocity and attenuation measurements of homogeneous and inhomogeneous liquids, as well as to measure their backscattering properties by changing the transducer type to a focalized one.
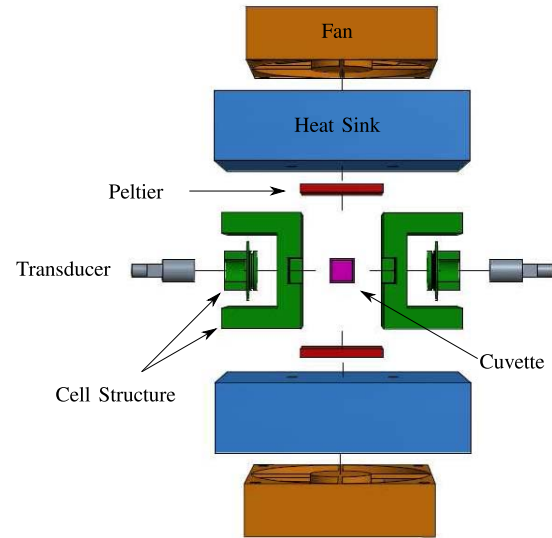

Fig. 1. Spectrometer (exploded top view). The structure is made of aluminum and contains a cavity for insertion of a commercial cuvette. Two transducers operate in through-transmission mode and are positioned at two opposite faces of the cell. At the other two faces of the cell, the Peltier cells, heat sinks, and fans are positioned for thermal control. As a reference, the cuvette has 12.5 mm × 12.5 mm external dimensions.

This paper is ordered as follows. The spectrometer design and theoretical operation principles are presented in Section II. Section III-A describes several tests to show the fundamental features of the instrument related to thermal control, precision, and repeatability. Results of propagation velocity and attenuation of aqueous NaCl solutions and dispersive liquids (castor and silicone oils) follow in Sections III-B and III-C, respectively, to demonstrate the applicability of the system in the measurement of small and higher attenuation samples. Finally, Section III-D presents an application of the spectrometer in backscattering of polystyrene particle suspensions.

II. SPECTROMETER

Fig. 1 shows an exploded top view of the spectrometer. Its main structure is constructed from an aluminum block, which is a good thermal conductor and easy machining material. A commercial spectrophotometer-type cuvette made of quartz, glass, or polymer material, which contains the liquid sample of interest, is placed in a rectangular cavity at the center of the block. The cuvette can be easily removed from the spectrometer to change the liquid of interest. This is one of the most important characteristics of this spectrometer, because when the sample is directly placed in a sample chamber, cleaning is in general a complicated matter, especially when dealing with viscous samples or which contain particles or microorganisms, for example. Therefore, one can either clean or sterilize the cuvette or simply use disposable ones, making sample manipulation much easier.

Two ultrasonic transducers are placed at two opposite faces of the aluminum block, parallel to the cuvette walls. They can operate in pulse-echo or through-transmission mode and, as the cuvette must be removed from the spectrometer to change samples, they cannot touch the cuvette walls. Then, a solution for acoustic coupling is to use a thin layer of

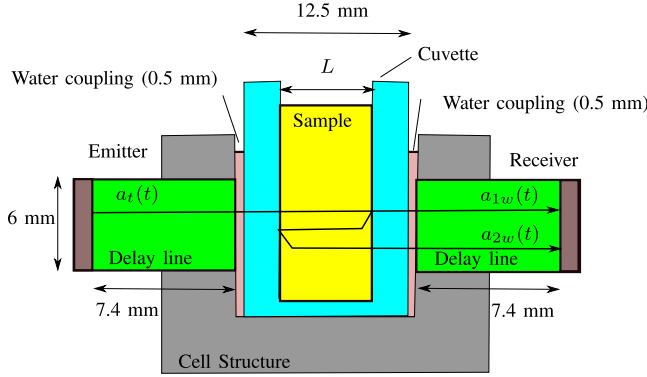Fig. 2.   Photograph of the polystyrene thermal box and spectrometer.

Fig. 3.   Cross section of the spectrometer. The transmitted signal $a_t(t)$ propagates through all layers and reaches the receiver as signal $a_{1w}(t)$, in the case of water sample. A multiple reflection inside the cuvette is received as signal $a_{2w}(t)$ and is used to measure the sample length L. The main dimensions are shown (not to scale), and cuvette height is 42 mm. The value of L can be varied according to the cuvette (10, 4, or 2 mm, for example).

distilled water (0.5 mm), which is an adequate coupling material due to its low attenuation and known properties.

Thermoelectric Peltier cells are used to control the block/sample temperature and are attached to the other two faces of the aluminum block, using heat sinks and coolers to improve heat exchange between the spectrometer body and ambient. A platinum resistive temperature sensor (Isotech, model 935-14-61, NIST traceable calibrated, accuracy of 3 mK) is positioned inside the cuvette containing the sample of interest, and signal conditioning and acquisition are made by a high-precision reference temperature monitor (Isotech, model Millik, resolution of 0.1 mK, and accuracy 3 mK). An electronic circuit was designed to drive the Peltier cells and a digital proportional–integral–derivative controller was implemented in real time. To improve thermal stability, the spectrometer is placed inside a polystyrene box where the air temperature is controlled by a similar Peltier-based system. A magnetic stirrer is placed at the bottom of the block to homogenize the sample temperature inside the cuvette and enhance stability in inhomogeneous liquids. Fig. 2 presents a picture of the polystyrene box and the spectrometer inside it.

A. Principle of Operation

A cross section of the spectrometer, with dimensions of acoustic interest (not to scale), is shown in Fig. 3. For the measurement of propagation velocity and attenuation,

a relative through-transmission technique, using distilled water as reference, is used. In a first calibration step, the cuvette is filled with distilled water to measure the sample length as a function of temperature T, $L(T)$. The transmitter is excited with a short pulse and the first direct signal propagates through all layers (silica delay line, water coupling, cuvette walls, and sample), reaching the receiver as signal $a_{1w}(t)$. The time delay between $a_{1w}(t)$ and the first multiple reflection inside the cuvette, $a_{2w}(t)$, is used to measure the sample length $L(T)$. In a second step, distilled water is changed by the liquid sample of interest and the direct signal, which is then called $a_{1\ell}(t)$, is measured for the calculation of the acoustic parameters. These steps are detailed in the following paragraphs.

1) Calibration Process: When using distilled water as reference, the direct signal and the first reflection inside the cuvette are called $a_{1w}(t)$ and $a_{2w}(t)$, respectively, and can be represented in the frequency domain as

$$A_{1w}(f) = K_{1w}A_t(f)e^{-\alpha_w(f)L}e^{j\phi_{1w}(f)} \qquad (1)$$

$$A_{2w}(f) = K_{2w}A_t(f)e^{-\alpha_w(f)3L}e^{j\phi_{2w}(f)} \qquad (2)$$

where K_{1w} includes the transmission coefficients between the emitter and receiver transducers, as well as the attenuations in the delay line, water coupling, and cuvette walls, $K_{2w} = K_{1w}.R_{wc}^2$, where $R_{wc} = (Z_c - Z_w)/(Z_c + Z_w)$ is the reflection coefficient between the sample of distilled water (subscript w) and the cuvette material (subscript c), $Z = \rho \cdot c$ is the acoustic impedance, ρ is the density, c is the propagation velocity, $A_t(f)$ is the spectrum of the transmitted signal, $\alpha_w(f)$ is the attenuation coefficient in water, f is the signal frequency, $\phi_{1w}(f) = -2\pi f(\Delta t_b + \Delta t_w)$ is the spectral phase of $a_{1w}(t)$, $\phi_{2w}(f) = -2\pi f(\Delta t_b + 3\Delta t_w)$ is the spectral phase of $a_{2w}(t)$, Δt_w is the propagation delay across the length L in water, and Δt_b is the propagation time from emitter to receiver, disregarding the sound propagation delay in the sample.

As propagation velocity in water, $c_w = L/t_w$, is tabulated as a function of temperature [26], the value of L can be obtained from the difference of the spectral phases of the first and second echoes [27]

$$\phi_{1w}(f) - \phi_{2w}(f) = 4\pi f \Delta t_w = 4\pi f \frac{L(T)}{c_w(T)} \qquad (3)$$

which results in

$$L(T) = \frac{\phi_{1w}(f) - \phi_{2w}(f)}{2\pi f} \cdot \frac{c_w(T)}{2} \qquad (4)$$

while the attenuation coefficient (in Np/m) is calculated from the relationship between the spectrum magnitudes of the first and second echoes

$$\frac{|A_{1w}(f)|}{|A_{2w}(f)|} = \frac{K_{1w}}{K_{2w}}e^{\alpha_w(f)2L(T)} = \frac{1}{R_{wc}^2}e^{\alpha_w(f)2L(T)}. \qquad (5)$$

It should be noted that the attenuations in the delay line, water coupling, and cuvette walls are canceled in this relationship, and attenuation in water results

$$\alpha_w(f, T) = \frac{1}{2L(T)}\ln\frac{|A_{1w}(f)|}{|A_{2w}(f)|} + \frac{1}{L(T)}\ln R_{wc}. \qquad (6)$$

At the end of the calibration process, the value of temperature T, path length $L(T)$, and the first echo $a_{1w}(t)$ is stored to calculate the properties of other liquids, which are described in the following step.

2) Measurement Process: After the calibration process, the water in the cuvette is replaced by the liquid of interest. This can be done by using the same cuvette (after proper cleaning), or another similar cuvette. In the second case, each cuvette can have its own calibration data, which results in increased accuracy but is more time-consuming. Otherwise, one could rely on a unique calibration data for different cuvettes, which would result in faster measurements but lower accuracy due to random geometrical variations from different cuvettes. However, this would be the best approach when using disposable cuvettes, for example. These two cases are evaluated in Section III-A.

When the cuvette is filled with the liquid sample of interest, the first direct signal is named $a_{1\ell}(t)$, whose spectrum is

$$A_{1\ell}(f) = K_{1\ell}A_t(f)e^{-\alpha_\ell(f)L}e^{j\phi_\ell(f)} \qquad (7)$$

where $K_{1\ell}$ includes the transmission coefficients between the emitter and receiver transducers, as well as the attenuations in the delay line, water coupling, and cuvette walls, $\alpha_\ell(f)$ is the attenuation coefficient in the liquid sample, $\phi_\ell(f) = -2\pi f(\Delta t_b + \Delta t_\ell)$ is the spectral phase, and Δt_ℓ is the propagation delay across the liquid.

The phase velocity in the liquid as a function of frequency and temperature, $c_{p\ell}(f, T)$, can be calculated from the difference between the spectral phases of the reference signal (distilled water), $\phi_{1w}(f)$, and of the liquid of interest, $\phi_\ell(f)$

$$\begin{aligned} \phi_{1w}(f) - \phi_\ell(f) &= 2\pi f(\Delta t_\ell - \Delta t_w) \\ &= 2\pi f\left[\frac{L(T)}{c_{p\ell}(T)} - \frac{L(T)}{c_w(T)}\right] \end{aligned} \qquad (8)$$

where $c_w(T)$ is the phase velocity in distilled water as a function of temperature. The phase velocity in the liquid as a funcion of temperature is

$$c_{p\ell}(f, T) = \frac{c_w(T)}{1 + \left[\frac{\phi_{1w}(f) - \phi_\ell(f)}{2\pi f}\right] \cdot \frac{c_w(T)}{L(T)}}. \qquad (9)$$

Attenuation coefficient in the liquid sample (in Np/m) can be calculated from the relationship between the spectrum magnitudes of the reference signal, $|A_{1w}(f)|$, and of the signal that crossed the liquid of interest, $|A_{1\ell}(f)|$

$$\frac{|A_{1w}(f)|}{|A_{1\ell}(f)|} = \frac{K_{1w}}{K_{1\ell}}\frac{e^{-\alpha_w(f,T)L(T)}}{e^{-\alpha_\ell(f,T)L(T)}} \qquad (10)$$

which results in

$$\alpha_\ell(f, T) = \alpha_w(f, T) + \frac{1}{L(T)}\ln\frac{|A_{1w}(f)|}{|A_{1\ell}(f)|} + \frac{1}{L(T)}\ln\frac{K_{1\ell}}{K_{1w}}. \qquad (11)$$

For most liquids, a power-law dependence for attenuation can be assumed [14], [28], [29]. In these cases, the frequency-dependent part of the attenuation coefficients (6) and (11) can be approximated by an expression of the kind $\alpha_0\ f^n$, where the exponent n is the power dependence, typically

between 1 and 2 for liquids. The values of α_0 and n can be obtained from a curve fitting of the experimental signals obtained from (6) and (11).

The term dependent on the transmission coefficients in (11) is

$$\frac{K_{1\ell}}{K_{1w}} = \frac{(Z_c + Z_w)^2}{(Z_c + Z_\ell)^2} \cdot \frac{Z_\ell}{Z_w} \qquad (12)$$

where Z is the acoustic impedance, the subscripts c, w, and ℓ refer to the cuvette, distilled water, and liquid sample of interest, respectively. In this relationship, the attenuations in the delay line, water coupling, and cuvette walls are canceled.

For distilled water, dispersion is negligible and a quadratic dependence with frequency is assumed for attenuation, that is, (6) can be approximated by $\alpha_w(f, T) = \alpha_{0w}(T)f^2$ [28], [30]. When working with low dispersion aqueous solutions (with similar properties to water), the ratio $(K_{1\ell}/K_{1w})$ has a small variation with frequency. In the determination of α_0 and n, the constant term as a function of frequency can be removed from the experimental data and then a curve fitting is performed. As a result, when the ratio (12) is constant with frequency, it does not have influence in the calculation of α_0 and n. As will be seen in the experimental results, in the case of oils with velocity dispersion, the ratio (12) will have a small variation as a function of frequency when compared to $\alpha_0\ f^n$.

B. Ultrasonic Instrumentation

Two high-frequency unfocused transducers [Olympus, model V2022(BC)] were used as transmitter and receiver. These transducers have a piezoelectric element diameter of 6 mm, a silica delay line of 2.5 μs (in pulse echo), central frequency of 75 MHz, and nominal -3-dB bandwidth of 59.8 MHz.

A programmable pulser-receiver (JSR, model DPR500, with remote pulser RP-H2, 500-MHz bandwidth) excites the emitter with a short pulse and amplifies the received echoes. When working in the frequency range of 100 MHz and higher frequencies, pulser and transducer should be connected by short cables, because due to impedances mismatches, the electrical excitation pulse can be reflected at the interfaces between the cable/transducer and cable/pulser. These reflections travel in the cable and excite the transducer again after a short period, changing signal in time and frequency domains, and the manufacturer recommends that the cables have lengths smaller than 30 cm to reduce this effect [31]. The received echoes are digitized by an acquisition board (Signatec, model PX14400, 14 bits resolution, max. sample rate 400 MHz) and postprocessed in MATLAB, which also controls the pulser settings, acquisition board, and thermal control system.

III. EXPERIMENTAL RESULTS AND DISCUSSION

Experimental results show the potential applications of the spectrometer, its thermal stability, measurement accuracy, and dynamic range. Aqueous mixtures of NaCl with concentrations ranging from 0.01% to 20% in weight (w/w), which have attenuations smaller than that of water, and samples of
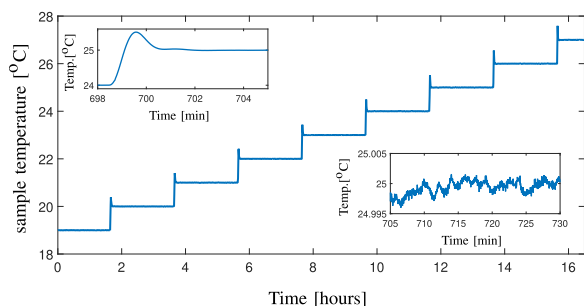
Fig. 4. Step responses showing sample temperature control and stability tests. The details show the transient and steady-state responses when there is a step from 24 °C to 25 °C.

higher attenuating oils, are used in the experiments. Finally, a backscattering experiment using polystyrene particles shows the versatility of the spectrometer to analyze particle sizes in suspensions.

A. Thermal Stability and Reproducibility

Thermal stability of the system was evaluated by using distilled water as sample and a 3-ml-volume quartz cuvette (Starna Scientific, external dimensions [mm] $12.5 \times 12.5 \times 45$ and internal dimensions 10×10). Fig. 4 shows the results of sample temperature when steps are applied to the system. The inset of Fig. 4 displays the transient and steady-state responses when the temperature was changed from 24 °C to 25 °C, at 698.5 min. Stabilization within 0.01 °C occurred in 4 min, while stabilization within 0.005 °C took 6 min.

In a long-term test, the spectrometer temperature was set to 25 °C and the thermal box temperature was set to 24 °C. This setpoint was kept constant for 20 h, and the sample temperature as a function of time presented a maximum variation of 0.005 °C with respect to the mean value. The acoustic signals were also recorded, and the maximum deviation in propagation velocity from the mean value of 1496.7 m/s was 2 cm/s. Attenuation measured at the frequency of 50 MHz resulted in a maximum variation of 0.01 dB/cm from the mean value.

These stability tests are important when one is interested in measuring a change in sample characteristics as a function of time, e.g., in process monitoring and microbial growth [19]. In these applications, temperature stability plays a fundamental role in system accuracy and precision, to detect small variations in sample properties. The temperature sensor is positioned 5 mm above the ultrasonic path, and small temperature gradients are expected inside the cuvette, but the temperature at the ultrasonic path is also kept constant within 0.01 °C.

Another case of interest occurs when several samples have to be measured and the cuvette must be removed from the spectrometer. There are circumstances where the same cuvette can be cleaned/sterilized and used again. This procedure guarantees the calibration and consequently more accurate results are obtained. When different samples have to be characterized, the use of a different cuvette for each sample makes the measurement process faster and easier. Nevertheless, uncertainty increases due to tolerances in cuvette's dimensions and alignment, as one calibration data are used for different cuvettes.

A quartz cuvette was filled with distilled water and calibrated at 25 °C. The cuvette was then removed from the spectrometer, a water sample was changed using the same cuvette, and new measurements were taken as soon as the temperature reached a 0.01 °C deviation from the setpoint. After 10 sample changes, the maximum variations in propagation velocity and attenuation coefficient relative to the true value were 2 cm/s and 0.01 dB/cm, respectively. This test was repeated for a polystyrene cuvette (BrandTech, external dimensions [mm] $12.5 \times 12.5 \times 45$ and internal dimensions 10×10) and the maximum variations in propagation velocity and attenuation coefficient were 8 cm/s and 0.05 dB/cm, respectively.

In another test, the calibration was performed for only one polystyrene cuvette and, for every sample change, a new cuvette (from the same batch) was used. This procedure is useful when several different samples have to be measured and sample changes must be faster, or when it is inadequate to clean/sterilize the cuvette, for example. In this case, the variations in propagation velocity and attenuation coefficient were considerably larger: 20 cm/s and 0.1 dB/cm, respectively (10 cuvettes). This test was not made for quartz cuvettes, because there was not a sufficient quantity of cuvettes, but this procedure is more likely to be done when using disposable ones.

The better performance of the quartz cuvette is expected when compared to the polystyrene ones, due to the lower attenuation and manufacturing tolerances in the former ones. Cuvette manufacturing quality can also have consequences in alignment errors relative to the transducers, and the higher acoustic attenuation in polystyrene reduces the bandwidth and frequency range to 15–35 MHz. On the other hand, polystyrene cuvettes have some advantages, because they are not so fragile, have lower cost, can be disposable, and can be the choice when larger uncertainties are allowed.

B. Water and NaCl Mixtures

Water and NaCl mixtures were used to evaluate resolution in the measurement of propagation velocity and attenuation coefficient. These aqueous solutions are interesting samples to be studied because these ions are very common in several areas (food science, biology, and medicine), the components are easily found, have low cost, the mixtures are relatively easy to prepare and chemically stable. Millero *et al.* [32] studied several aqueous electrolytes solutions and obtained equations describing the relationship between the propagation velocity and mass fraction of solute as a function of temperature in a concentration range from 1.4% to 26.4% and temperature from 25 °C to 95 °C. Measurements of low concentrations (less than 1% w/w) and attenuation data in water-NaCl samples were not found in the literature, which can be valuable in the calibration of ultrasonic systems, and this is another contribution of this work.

The samples prepared in this work were divided into two groups of high and low concentrations. The high concentration group contains samples with mass fraction from 1% to 20%, similar to the range chosen in [32]. Samples were prepared in mass fraction using distilled water, NaCl with
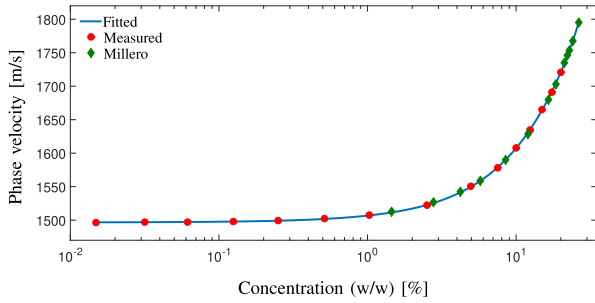
Fig. 5.   Phase velocity at 50 MHz and 25 °C as a function of concentration of NaCl. Type A uncertainties are smaller than 0.08 m/s.

TABLE I

POLYNOMIAL COEFFICIENTS FOR PHASE VELOCITY AND COEFFICIENT α_0 IN WATER NaCl MIXTURES

Coefficient	Phase Velocity [m/s]	$\alpha_0 \times 10^{-15}$ [dB s^2 cm^{-1}]
p_0	1496.656	1.861
p_1	9.947	-3.438×10^{-2}
p_2	2.041×10^{-1}	2.145×10^{-3}
p_3	-1.092×10^{-2}	-6.905×10^{-5}
p_4	1.958×10^{-4}	-6.553×10^{-8}

99.5% purity (Emfal), and an analytical balance (Shimadzu, ATX224, resolution 0.1 mg). The low concentrated dilutions were prepared by mixing 10 ml of the sample of 1% concentration with the same volume of distilled water and repeating the procedure with the obtained dilution to get smaller concentrations.

All mixtures were agitated for 10 min in a magnetic stirrer and rested for 1 day in closed bottles before performing the ultrasonic measurements. The mass fractions were checked from their density values [33] using a commercial densimeter (Anton-Paar, model DMA 4500, resolution 10^{-5} g/cm^3, thermal control resolution 0.01 °C).

Ultrasonic measurements using a quartz cuvette were made at 25 °C. Diffraction losses were under 0.05 dB in the worst case (maximum concentration and 20 MHz), and consequently, they were neglected due to the small values in comparison with the absolute attenuation values (around 4 dB for 1-cm propagation path in water).

These aqueous samples exhibit no significant dispersion and the phase velocities at the frequency of 50 MHz and 25 °C as a function of concentration are presented in Fig. 5. The circles represent the mean values obtained from triplicate measurements and Type A evaluation of uncertainty was employed [34], resulting in the maximum uncertainty of 0.08 m/s. The results for higher concentrations are very close to the ones presented by Millero et al. [32], presenting the maximum relative differences of 0.1%. The propagation velocity as a function of concentration w can be estimated from a fourth-order polynomial $\sum_{n=0}^{4} p_n w^n$, where the coefficients p_n are given in Table I.

Dispersion in pure water and in aqueous NaCl solutions is negligible or very small. As described in [28], velocity dispersion in water, in a frequency range from 0.3 to 10 MHz, is in the order of 4 cm/s, as predicted by Kramers–Kronig relations. However, it is difficult to measure such small variations in phase velocity as a function of frequency with accuracy,
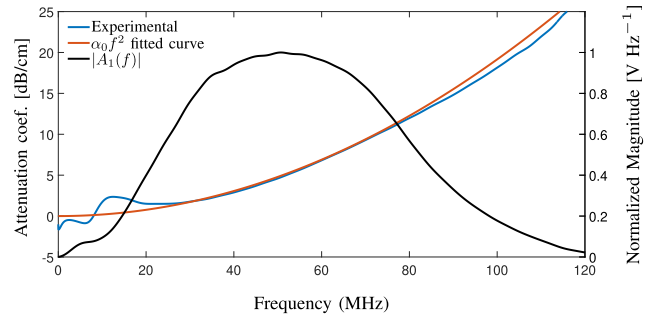

Fig. 6.   Attenuation coefficient as a function of frequency for water and NaCl mixture (20% w/w). Experimental and $\alpha_0 \ f^2$ fit curve. The normalized magnitude spectrum of signal $a_1(t)$ is also shown.
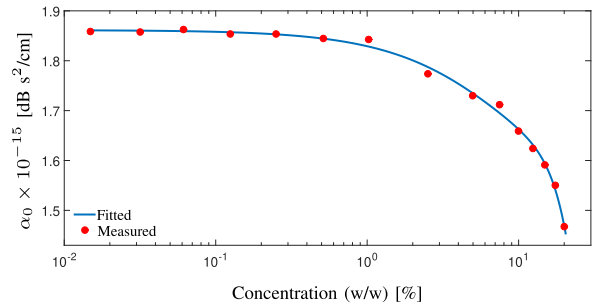

Fig. 7.   Value of α_0 as a function of concentration for water-NaCl mixtures at 25 °C. Maximum uncertainty is 0.01×10^{-15} dB s^2 cm^{-1}.

because they are in the same order of magnitude of the measurement uncertainties in this work. For nondispersive liquids, the power-law dependence of attenuation as a function of frequency can be well approximated by an expression of the kind $\alpha(f) = \alpha_0 \ f^2$. The last term in (11) depends on the acoustic impedances of cuvette, sample, and water, as expressed in (12). In the frequency range of operation, up to 100 MHz, these impedances are constant with frequency and contribute with a constant term as a function of frequency in the total attenuation coefficient. This term can be subtracted from the attenuation coefficient data before applying the curve fitting, which is frequency dependent.

Fig. 6 exhibits the experimental attenuation coefficient as a function of frequency for the 20% NaCl concentration and its corresponding curve fitting. The normalized magnitude spectrum of signal $a_1(t)$ is also shown in Fig. 6, where the −6-dB bandwidth is approximately from 20 to 80 MHz.

In Fig. 7, the values of α_0 as a function of concentration in the solutions are presented. Maximum uncertainty in α_0 coefficient was 0.01×10^{-15} dB s^2/cm. From these results, it can be seen that attenuation in these solutions is smaller than attenuation in pure water. The solid curve represents the α_0 value approximated by a fourth-order polynomial, whose coefficients are given in Table I.

Kurtze and Tamm [35] measured sound absorption in several electrolytes solutions, showing that 1-1-valent electrolytes (such as NaBr, NaCl, and KBr) have absorption smaller than that of water, due to the structure-changing influence of the solute on water. They also showed results for the absorption in aqueous NaBr solutions, which presented 70% of the water absorption value. As seen in Fig. 7, the case of 20% w/w NaCl
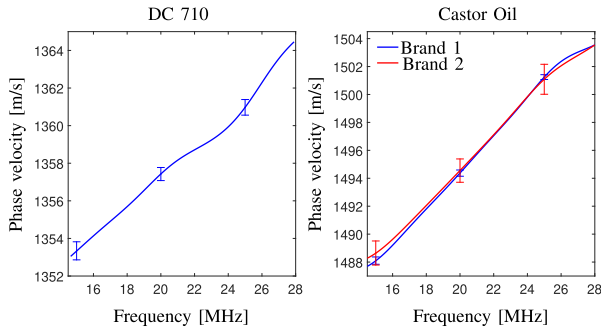
Fig. 8. Phase velocities in DC-710 silicone and castor oil at 23 °C as a function of frequency. Type A uncertainties were calculated for some frequencies and are smaller than 1 m/s.
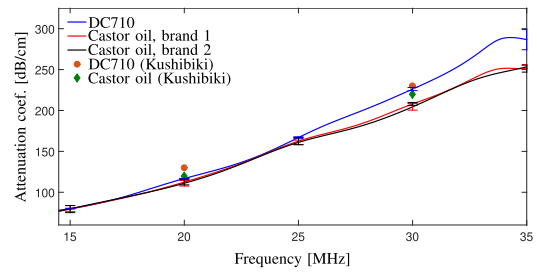

Fig. 9. Attenuation as a function of frequency for DC-710 silicone oil and castor oil at 23 °C. Maximum uncertainty is 5 dB/cm at the frequency of 30 MHz.

in water resulted in 79% of the absorption in pure water, so the results found in this work are in agreement with [35].

These measurements point out the capability of the system to measure propagation velocity and attenuation coefficient in dilute mixtures of NaCl in water. These samples have low cost and are easy to prepare and could be used in the calibration of ultrasonic measurement instrumentation for liquids.

C. Silicone and Castor Oil

Dow Corning DC-710 silicone oil and castor oil (two brands: RioQuimica and Vicpharma) were used as higher attenuation samples. DC-710 silicone oil was proposed as a reference for attenuation measurement in [36] and results for higher frequencies are available in [14] and [37]. Due to the higher attenuation in these oils, a quartz cuvette with 2-mm internal path was used instead of the one with 10 mm.

These samples show dispersion, and the corresponding phase velocities at 23 °C as a function of frequency are shown in Fig. 8, from 14 to 28 MHz. Maximum uncertainty was estimated as 1 m/s, which is due to errors in alignment, which is significantly higher in these cuvettes with shorter paths. The smaller frequency range in comparison with the aqueous solutions results is due to the higher attenuation in these oils. At 20 MHz, the phase velocity values for silicone oil and castor oil obtained in this study were 1358 and 1494 m/s, while in [14] were around 1370 and 1550 m/s, resulting in relative differences of 0.8% and 3.6%, respectively. The velocity dispersions for silicone oil and castor oil were 0.8 and 1.3 (m/s)/MHz in this work and 0.36 and 0.4 (m/s)/MHz in [14]. Szabo [28] presents the following results of velocity dispersions for silicone oil and castor oil: 0.4 and 0.7 (m/s)/MHz, respectively, which were measured from 1 to 10 MHz. These differences could be related to variations in some oil constituents, for example, feedstock, aging, or degradation. In [36], long-term stability tests were conducted for attenuation in DC-710 silicone oil, but not for propagation velocity, which is more sensitive to variations in sample composition than attenuation.

Attenuation coefficients as a function of frequency are shown in Fig. 9, where the symbols are values obtained from [14], and maximum uncertainty was 5 dB/cm at the frequency of 30 MHz. Attenuation coefficient in these samples does not obey a quadratic power law, and for DC-710, the attenuation curve was fit by $\alpha(f) = 5.46 \times 10^{-11} f^{1.68}$ dB/cm. Kushibiki *et al.* [14] did not explicitly

calculate the value of α_0 but verified a decrease in the value of n as a function of frequency, starting from 1.6 at 20 MHz until 1.3 at 200 MHz (at 23 °C). Zeqiri [36] obtained $\alpha(f) = 1.88 \times 10^{-11} f^{1.79}$ dB/cm, where the experiments were made at 10 MHz and 20 °C. The value of n obtained in this work is in agreement with both references, and the absolute value of attenuation is close to the ones reported in [14] and [36]. The larger difference for silicone oil, at the frequency of 20 MHz, is 10%.

For the samples of castor oil, the attenuation coefficient was approximated by $\alpha(f) = 3.47 \times 10^{-11} f^{1.68}$ dB/cm. Kushibiki *et al.* [14] obtained a value of $n \sim 1.6$ at 20 MHz, with decreasing values for higher frequencies (1.3 at 200 MHz), while Dunn and Breyer [37] obtained $n = 1.66$ at 30 °C (measured between 30 and 100 MHz). Therefore, the values of absolute attenuations are also close to the ones obtained by other authors, pointing out that the system is able to measure low and high attenuations.

These samples exhibit velocity dispersion, and a comment about the contribution of the ratio (12) in attenuation should be made. By considering castor oil, density is 950 kg/m³, and propagation velocity varies from 1408 and 1504 m/s, in the frequency range between 15 and 28 MHz. The quartz cuvette has $L = 2$ mm and impedance Z_c equal to 12.1 MRayl, while water impedance Z_w is 1.5 MRayl at 25 °C. Then, the last term in (11) varies between −1.5 and −1.7 dB/cm, which represent a variation of 0.2 dB/cm, much smaller than the total attenuation coefficient that varies between 100 and 200 dB/cm in this frequency range. Then, for these samples, the contribution of the ratio (12) varies with frequency but is small and practically constant when compared to the variation of $\alpha_0 f^n$.

D. Backscattering

Using the same spectrometer concept, a focused transducer (Olympus, model V390-SU/RM, 50 MHz, focus at 12.7 mm, focus diameter 64 μm) was used in the pulse-echo mode in backscattering experiments using aqueous suspensions of polystyrene particles. When using a cuvette as a sample holder, there are multiple reflections at the several interfaces due to impedance mismatch and also signal attenuation at cuvette walls. However, if temperature is kept constant, these reflections remain unchanged and a reference signal can be stored, related to propagation in the continuous medium without particles.

In relation to the cuvette material, although a polystyrene one would be better in terms of acoustic impedance, the higher
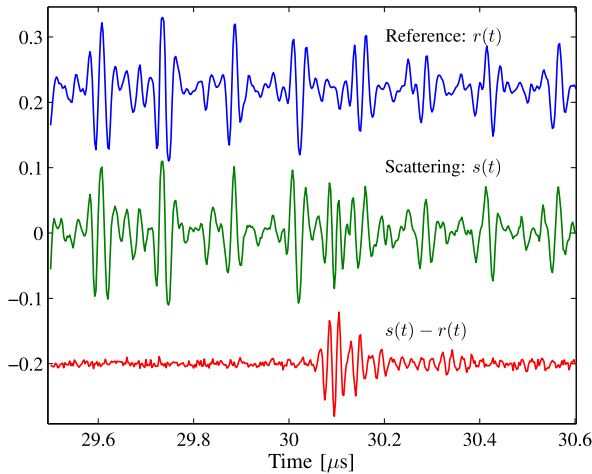
Fig. 10.   Backscattered signals (in volts): reference signal $r(t)$ acquired with a distilled water sample (top) and a backscattered signal $s(t)$ containing particle reflections of polystyrene particles with 15 μm diameter. These signals contain the multiple reflections at the cuvette walls. The subtraction between the two previous signals (bottom) highlights the particle backscattered echoes.

attenuation does not allow proper visualization of the particles echoes, so glass cuvettes (Hach, external dimensions [mm] 12.5 × 12.5 × 45 and internal dimensions 10 × 10) were chosen for the experiments.

A reference signal $r(t)$ was acquired at 25 °C, using distilled water as a sample (continuous medium). This signal contains echoes produced at the interfaces between the sample and cuvette walls, and it is assumed that, as long as temperature is kept constant, these echoes do not change. Then, 1 μL of a suspension of spherical polystyrene particles with 15-μm diameter and 10% in mass (Sigma Aldrich) was added to the cuvette, resulting in approximately 20 particles per microliter in the suspension. The suspension was gently mixed and, to avoid particle settling, a magnetic stirrer was used during the experiments, then signals containing backscattering echoes $s(t)$ were acquired.

Due to the low particle concentration, it was possible to notice when particles crossed the transducer focus and reflected the acoustic signal. These particle echoes could be detected among the several fixed interface echoes and were random in amplitude and time occurrence. Approximately, 2000 signals were acquired sequentially and, from this set of signals, about 20% presented particle reflections. As an approximation to obtain the waveform containing only particle scattering, the echoes produced by reflections at sample/cuvette wall interfaces were removed from the backscattered echo, by performing a simple subtraction. Fig. 10 shows the reference signal $r(t)$ acquired with a distilled water sample (top), a backscattered signal $s(t)$ containing particle reflections (middle), and the subtraction between the two previous signals $s(t) - r(t)$ (bottom), from which the backscattered signal is highlighted from the multiple interface reflections.

Then, the particle echoes were windowed to isolate the signal of interest and reduce noise, and the experimental backscattering spectra were compared to a theoretical model [38], [39] that describes the particle backscattered acoustic pressure as a function of frequency. This function, in spherical coordinates,
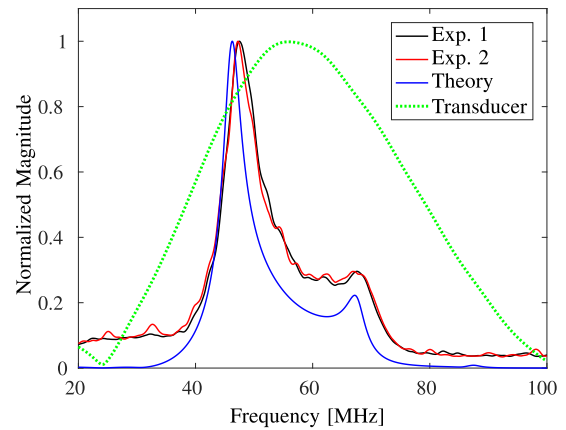

Fig. 11.   Backscattered spectrum: theory and experiment for 15-μm-diameter polystyrene particles. The transducer spectrum is also shown.

is described by

$$P_{\text{scat}} = -P_{\text{inc}} \sum_{m=0}^{\infty} i^m (2m+1) D_m P_m(\cos\theta) h_m(k_f f) e^{i\omega t} \quad (13)$$

where P_{inc} is the incident pressure at the particle, which is located at (r, θ, ϕ), m is the order, P_m are the Legendre polynomials, h_m are the Hankel functions, k_f is the fluid wavenumber, and D_m is defined by

$$D_m = \frac{Z_f j_m'(k_c a) j_m(k_f a) - Z_p j_m'(k_f a) j_m(k_p a)}{Z_f j_m'(k_c a) h_m(k_f a) - Z_p h_m'(k_f a) j_m(k_p a)} \quad (14)$$

where the subscripts f and p refer to fluid and particle, respectively, $Z = \rho c$ is the acoustic impedance, ρ is the density and c is the propagation velocity, j_m are the Bessel functions of the first kind, $'$ is the derivative with respect to the argument, and a is the particle radius.

For polystyrene particles in water and assuming that the particles are at the transducer focus (12.7 mm): $\theta = \pi$ rad, $a = 7.5$ μm, $\rho_f = 997$ kg/m^3, $c_f = 1496$ m/s, $\rho_p = 1050$ kg/m^3, $c_p = 2350$, and 1130 m/s (longitudinal and shear). The results for 15-μm-diameter particles are presented in Fig. 11: transducer spectrum (in pulse echo), theoretical, and experimental results, achieved from two independent sample preparations.

The transducer response was obtained from the delay line/water coupling reflection, which is centered at 55 MHz and was used as an approximation of function P_{inc}. Attenuation coefficient in water was also considered in P_{scat} expression because it is significant in this frequency band. Two of the peaks predicted by the theory, which are related to particle resonances induced by the incident field, were detected at the frequencies of 46 and 67 MHz. There are higher frequency peaks that fall outside the frequency band of the transducer, and they did not produce backscattered signals above the noise level. There is a good agreement between the experimental and theoretical results, and the wider experimental spectrum is due to small variations in particle diameters and also to signal windowing that produces spectral leakage.

The experiment was repeated with particles of 10-μm diameter, and the results are shown in Fig. 12. As a result of the smaller particle diameter, the first resonance was found
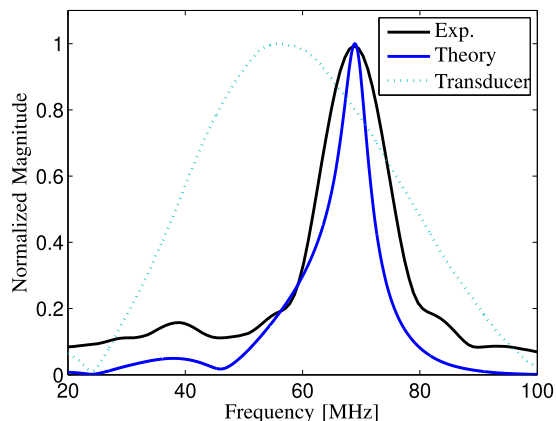
Fig. 12. Backscattered spectrum: theory and experiment for 10-μm-diameter polystyrene particles. The transducer spectrum is also shown.

at 69 MHz, and again there was a good agreement between the theory and the experiment. Higher resonances were out of the transducer band and could not be detected.

IV. Conclusion

In this work, a versatile spectrometer for liquids was proposed, which gathers important characteristics in the same instrument: ease of sample handling by employing commercial cuvettes (disposable or sterilizable); operation with small volumes (≤ 3 ml) and variable sample path length, determined by the cuvette; dry, precise, and accurate thermal control; and pulsed, wide bandwidth operation (20–80 MHz). The use of disposable or sterilizable commercial cuvettes is a significant advantage when the samples of interest are biological or viscous, e.g., facilitating their cleaning and manipulation. The spectrometer presents high accuracy, with errors of 2 cm/s (0.001%) in propagation velocity and 0.01 dB/cm (0.2%) in attenuation, and thermal stability better than 0.01 °C (when measuring distilled water, which has reference values for comparison). The system was used to measure acoustic parameters of low attenuation liquids, like aqueous NaCl solutions, with attenuation of the order of 5 dB/cm at 50 MHz. These results can be used for calibration of ultrasonic systems for the characterization of liquids because these samples have low cost, easy to prepare, and stable. On the other hand, the system is also capable to measure more dispersive and viscous media, with attenuation coefficients up to 300 dB/cm. In the case of aqueous NaCl solutions, we can talk about relative differences from other works, and the maximum relative difference was 0.1% for propagation velocity (there is no attenuation data for comparison). In the case of oils, the maximum relative differences for propagation velocity were 4% and 10% for attenuation coefficient. In addition, there is also the possibility of using the same spectrometer concept in backscattering studies, by simply changing the transducers to focused ones.

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