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Compressibility of Nano Inclusions in Complex Fluids by Ultrasound Velocity Measurements

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Abstract—We present a high precision ultrasonic velocimeter for a small volume sample (1 cm³) for a path length of 1 cm achieved. The method used is based on the time of flight measurement with an original signal processing technique: the barycenter method. With our system, we have measured the sound velocity with an accuracy of $10^{-5}$. The detection of a difference in velocity between two liquids of about 2 cm/s is achieved. The compressibility of the reference liquid can then be deduced with an accuracy better than 0.1%. Using this custom-made system, density and ultrasonic velocity of the medium. The ultrasonic measurements of velocity are carried out by methods either temporal or spectral [3], leading to an accuracy of about $10^{-5}$ to $10^{-6}$ for difference measurements of a volume around 1 mL. The temporal method consists in measuring the time of flight between the emission of a short duration signal and its reception either by transmission or by reflection. The process to determine the time of flight uses either zero-crossing of the signal or the search for a maximum. An alternative method consists in starting the emission when a signal is detected in transmission. In this method, known as the Sing Around Method [4], time is determined by the measurement of the impulse repetition period. Taking into account multiple reflections, this method has to deal with trigger problems especially in localized

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\[ \beta = \frac{1}{\rho u^2}. \] (1)

According to this relation, the compressibility can be determined from two measurements: the measurement of the medium density, \( \rho \), and the measurement of the sound velocity, \( u \), of the same medium. In our experiment, the solution consists of an organic solvent with inclusions. Such inclusions may be reverse micelles, macromolecules, or proteins, encapsulated in micelles or in an aqueous solution. While compressibility of the solution may easily be obtained, the compressibility of inclusions must be deduced.

Consequently, to obtain the compressibility of the inclusions dispersed in a solvent, we need to know

(i) the compressibility of the solvent, which can be obtained by measurements of the oil density \( \rho_{\text{oil}} \) and the ultrasonic velocity of the solvent \( u_{\text{oil}} \) [see (1)];

(ii) the compressibility of the solution, which can be determined from measurements of the density \( \rho \) and the ultrasonic velocity \( u \) of the solution; and

(iii) the volume fraction, \( \phi_{\text{inc}} \), calculated using (4).

The accuracy on the compressibility of inclusions at low concentration depends primarily on the accuracy of \( \rho \) and \( u \).
mally regulated at 25 ± 0.01°C (Huber HS 40). Furthermore, the unit is placed in a temperature-controlled room. A permanent temperature control is monitored by measurement of the ultrasonic velocity in the reference cell. The first cell is filled with the reference liquid (solvent) and the second cell with the solution. The difference in velocity between the two cells represents the speed of sound variation, induced in the solvent by the inclusions.

Each cell comprises two identical lithium niobate piezoelectric transducers. They are glued on stainless steel elements, the parallel faces of which are optically polished (Fig. 1). These elements ensure the impedance matching between the transducer and the medium. They are essentially to damp the signal and thus to improve temporal resolution. The thickness of the steel elements is calculated to avoid multiple reflections and interference with the desired experimental signals. These elements introduce a delay, which must be taken into account during the calibration procedure. Hence, they are referred to as delay lines. The transmitting transducer emits an acoustic wave into the medium, and the receiving transducers convert the received pressure into a voltage. The excitation signal sent to the two transmitting transducers is a modulated coherent pulse at the resonance frequency of the transducers. The signal duration is equal to exactly \( n \) cycles (\( n \) is an integer) of the modulating signal, and consequently, does not yield discontinuities at the beginning or end of the impulse. The emitted signal is optimized first by digital simulation and then by experiment. Several features of the optimized are

(i) the modulating signal frequency corresponds to the center frequency of the transducers (\( f = 8 \) MHz) in order to optimize energy transfer;
(ii) the duration of the modulated impulse is equal to 2 cycles of the modulating signal (\( n = 2 \)), and is a compromise between sufficient energy being received and too large of a measurement bias;
(iii) the delay between two consecutive emissions (1 ms) is selected in order to avoid interactions of multiples echoes; and
(iv) the amplitude of emitted signal (10 V) is optimized with respect to the signal-to-noise ratio as well as avoid an increase in temperature of the solution.

After propagation in the medium, the two signals provided by the two receiving transducers are sampled simultaneously (at 10 giga-samples per second) by a digital oscilloscope (model Lecroy 9424E) synchronized by a pulse generator. Given the total time of flight (medium plus delay lines), the temporal sampling resolution provides a theoretical accuracy to better than \( 10^{-5} \). The accuracy, which is limited by the sampling rate of our oscilloscope, can be easily improved by the following procedure. The received signals are averaged by the oscilloscope over 100 successive acquisitions, in order to improve the signal-to-noise ratio: this amounts to a measurement every 45 s. An overall average is also calculated on the result of 10 time of flight values. Fig. 2 provides the schematic diagram of the experimental setup.

A PC computer controls the data acquisition on via an IEEE 488 interface and the signal processing described below by home-made software (written in C language).

Considering the received signal profile, the precise time of the received wave beginning is difficult to determine. Moreover, the zero-crossing is perturbed by noise and the solution averaging of acquisitions is not a sufficient solution. To overcome this difficulty, the time of flight measurement is calculated by an integration of the received signal using the following relation

\[
t_{\text{vol}} = t_0 + \sum_{i} t_i a_i^2 / \sum_{i} a_i^2
\]

where \( t_{\text{vol}} \) is the time of flight, \( t_0 \) is the delay between the emission and the beginning of the analysis window, and \( t_i \) is the time and \( a_i \) is the \( i^{th} \) sample of the signal at the time \( t_i \). Eq. (9), by which one calculates the energetic gravity center, is known as the barycenter method. It will be noted
that the method introduces a bias, which is compressed by
the velocity difference determination.

D. Calibration

To obtain the time of flight in the medium, one first
has to measure the temporal shift introduced by the delay
lines. It is also necessary to know the length of the cell.
These two parameters lead to velocity \( u \). A calibration is
carried out in four steps for each cell. The measurement
cell is initially filled of standard liquid of known velocity
with a sufficiently high accuracy (1 cm/s). The velocity
must be close to that of the medium to explore, in order
to minimize the impedance mismatching. Second, the time
of flight, \( t_{dl} \), in the delay lines is measured in reflection.
The total time of flight, \( t_{tot} \), is then measured in transmis-
sion, and the time of flight in the medium, \( t_m \), is deduced
from the difference \( t_{tot} - t_{dl} \). Finally, knowing the velocity
of the medium and the time of flight, the length of the cell
\( d \) and, consequently, the variation of compressibility are
determined. The accuracy of the distance \( d \) is the sum of
the accuracy on the velocity of the standard liquid \( 10^{-6} \)
and on the time of flight in the medium (about \( 10^{-6} \)).
However, since \( d \) remains constant during a given experi-
ment, an error on its value does not generate errors on the
variation of velocity.

IV. EXPERIMENTAL RESULTS

A. Velocimeter Performance and Application to
Simple Fluids and Solutions

The measurement of the velocimeter stability in both
the short and medium term was investigated. Fig. 3 rep-
resents the evolution of the difference of time of flight be-
tween the two cells, using the zero-crossing method (a) and
the barycenter method (b) for 20 h. These curves clearly
demonstrate the advantages of the barycenter method with
respect to the classical method. In the barycenter calcula-
tion, the integration obviously reduces the noise. By the
barycenter method, the short-term measurements of the
time of flight (during a typical experiment of 5 h), gives a
standard deviation of 0.8 ns for one cell and 0.2 ns on the
difference between the two cells. These results correspond
to a velocity variation of about 1 cm/s (Fig. 4) over a ve-
locity of 1500 m/s. The accuracy thus obtained with our
velocimeter is thus about \( 10^{-5} \). The benefit obtained with
the barycenter method is 14 dB.

During the validation process, we have measured the
ultrasound velocity and density of methanol. The re-

tsults obtained are in excellent agreement with literature
[14]. In the same way, we have determined the com-
pressibility of a pure oil, isooctane. This value of \( \beta_{oil} =
121.0 \pm 0.2 \times 10^{-11} \text{ Pa}^{-1} \) has been used in (5). The stan-
dard deviation of this measure is lower than that given in
literature [15], [16].

Fig. 3. The time of flight difference between the two cells as a function
of time (over a 20 h period): (a) by the zero-crossing method and
(b) by the barycenter method (870 points of measurement). Zero-
crossing method: the standard deviation is 0.76 ns and barycenter
method: the standard deviation is 0.24 ns.

Fig. 4. Barycenter method: the time of flight difference (left axis) and
the velocity difference (right axis) between the two cells versus time
for 5 h. (110 points of measurement). The minimum is at -0.219 ns,
the maximum is at 0.173 ns, and the standard deviation is 0.083 ns.
In a second step, we have studied a protein (lysozyme of hen egg) solubilized in water. In a typical experiment, the reference cell contains water, while the measurement cell is filled with the protein solution. Fig. 5 illustrates the velocity variation between \( u_0 \) (velocity in water) and \( u \) (velocity in the solution) versus the protein concentration; in this experiment, the accuracy is better than 2 cm/s. Our results are in good agreement with those of literature: the slope of the curve \( (du/dC) \) is 0.259 \( \pm 0.001 \), compared with 0.257 [17]. This result corresponds to compressibilities of \( 4.70 \pm 0.5 \times 10^{-11} \) Pa\(^{-1}\) in our measurement and \( 4.67 \times 10^{-11} \) Pa\(^{-1}\) in the literature.

B. Application to Complex Fluids

In this last section, we present the measurement of the speed of sound and the compressibility of more complex fluids composed of 3 or 4 components. The studied fluid is composed of nano-droplets of water surrounded by a monomolecular film of a surfactant (sodium bis [2-ethylhexyl] sulfosuccinate: AOT) and dispersed in a solvent (isooctane); such a system is denominated reverse micelles [1]. The size of these spherical droplets is on the order of several nanometers and depends only on the water concentration, \( W_0 \) defined as the water-to-surfactant molar ratio. This parameter is experimentally controlled with accuracy. The droplets are thermodynamically stable [1].

In this experiment the reference cell contains also the pure solvent, while the measuring cell is filled with the micellar solution. The plot of the difference in velocity between the two liquids, versus \( W_0 \) is illustrated in Fig. 6. This velocity difference associated to density measurements enables us to determine the micellar compressibility (see Fig. 7). We observe an increase of the compressibility versus the number of water molecules per micelle, \( n_{H_2O} \) (logarithm scale). The accuracy obtained is around \( \pm 1 \times 10^{-11} \) Pa\(^{-1}\). Using the above-described velocimeter, we have been able to give a first estimate of the compressibility of the water in the vicinity of a membrane. We have found a value of \( 60.10^{-11} \) Pa\(^{-1}\), whereas bulk water yields a compressibility of \( 45.10^{-11} \) Pa\(^{-1}\). This confirms the unusual physical properties of water in contact with a membrane-mimetic system, which mirrors the properties of biological membranes [19]-[21].

When introducing an additional component to the system (a protein, cytochrome c, inserted into reverse micelles), the determination of the compressibility of the protein is still possible, although more difficult. The sensitivity and the accuracy of such measurements are illustrated in Fig. 8, where we have plotted the protein micellar solution compressibility versus protein volume fraction (\( \phi_p \)). These results show that, even under arduous experimental conditions, the values of compressibility remain measurable and precise for very small variations of the volume fraction of the protein.
Fig. 8. Compressibility of a micellar solution of cytochrome c at $W_0 = 8.2$ versus the protein volume fraction $\phi_p$ (error bars: ±0.05%).

V. SUMMARY

We have built a high accuracy apparatus to determine the compressibility of nanometric-size inclusions, dispersed at low concentration in a complex biomimetic system. The measurement of the sound velocity in the medium renders possible estimation of small differences in velocity between a reference liquid and the same liquid containing inclusions of interest. The velocimeter, precisely thermally regulated, also allows, the permanent temperature control of the tandem cells. The difference of velocity measurement reduces the influence of residual variations of the temperature. The method used is based on the time of flight measurement. In addition, we make use of an original signal processing technique: the barycenter method. As a consequence, the precise determination of the speed of sound becomes less sensitive to noise and the variance of the results is strongly decreased when compared with classical temporal methods. In particular, we have measured a reduction in the standard deviation of about 14 dB.

With the described system in hand, we have measured the sound velocity, in low volumes of solvent (around 1 cm$^3$) with an accuracy of $10^{-5}$. This accuracy permits the detection of a difference of about 2 cm/s in velocity between two liquids. The compressibility of the reference liquid can then be deduced with an accuracy of about 0.2%. In this report, we have been able to measure the compressibility of fluids of increasing complexity, composed of one to four different components, with excellent accuracy. The method has also been applied with success in our laboratory to micellar solutions of biological macromolecules, for example, to determine the compressibility of proteins at very low hydration levels and thus to reach the protein intrinsic compressibility [22].

REFERENCES