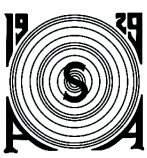

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Photoacoustical evaluation of thermal response of microbubbles

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Photoacoustic technique has emerged as a tool for the study of liquid, gel, suspension behaviour and has been recently employed in a number of new biomedical applications. A new photoacoustic sensor is presented, which was designed and realized to analysing photoacoustic signals from slotions filled with microbubbles, usually used as contrast agents in echotomography imaging. The device is a closed cell where photothermal expansion in acqueous solution samples causes the deflection of a thin membrane closing a short pipe 0.7 mm by radius. The overall set up acts as a Helmholtz resonator, where the solution in the pipe is the mass oscillating under the driving force produced by the alternating expansion of the solution in the cell body. Displacement of the smembrane was measured using a laser probe, whereas photoacoustic signal was generated by chopping a laser light beam impinging into the solutin through a glass window in the cell. The response of the cell filled with shelled microbubbles was investigated with respect to water behaviour, at the cell resonance frequency and for different temperatures**.**

1 Introduction

The use of microbubble contrast agents in diagnostic applications to medicine is a common practice nowadays for enhancing imaging contrast produced by the vascular structure of internal organs of human body, and therapeutical applications are in use, as well, for delivering drugs contained within the microbubbles at proper regions of the body [1, 2]. In both cases: whether the scattering efficiency of gas filled bubbles is exploited for diagnostic procedures, or the energy release of the radiating field is used for cavitating bubbles in therapeutics, ultrasound is commonly used. It's a priority of any methodology employed, therefore, the correct knowledge of the bubble-sound interaction, as it depends upon bubble radius, filling gas and shell material – if any -, that are to be properly investigated for any newly developed agent.

Common experimental investigations usually rely on the absorption/scattering efficiency of an ultrasonic pulse by microbubble solution vs. ultrasound frequency [3]; temperature dependence is also commonly pursued, since efficiency may be properly increased at body temperatures or temperature evaluation may be performed by ultrasound noninvasive methods, particularly in the case that the shell material undergoes a phase transition at the working temperatures.

The present paper describes a photoacoustic cell specifically developed for gas/liquid solutions characterization of the dynamical properties of shelled bubbles, as they affect the overall compliance of the solution, through the measurement of the vibration amplitude of a sensible membrane closing the cell.

2 The photoacoustic cell

The photoacoustic cell is a container of the solution, which is periodically illuminated by a laser beam that releases its energy to the solution and causes it to expand and contract periodically at the same frequency of the impinging light [4]. The volume variations of the solution are monitored through detection of the vibration amplitudes of a sensible membrane closing the cell.

The cell (see Fig.1) is a cylinder enclosure [5] 1,7cm by internal diameter, 5cm long, made of brass with one basis of quartz glass acting as the throughput window for a chopper laser light beam, that deposits electromagnetic energy to the solution at fundamental frequencies ranging from 100 to 2000Hz. A short pipe, 2.0cm long, 1.5mm by inner radius, is inserted in the lateral surface of the cylinder and is closed by a PVC thin membrane that follows the volume variations of the solution and whose movement is detected by an acoustooptical laser probe. A second pipe is also inserted in the lateral surface of the cylinder, through which one may fill and empty the cell with the solution to be measured. Between the two pipes, a small opening is produced on the lateral surface, which is closed with a removable lid, and it permits to evacuate undesirable air bubbles which could have been imprisoned in the cell. Inside of the cell, a thin helicoidal copper tube, 1mm inner diameter by size, 20cm long is set coaxial with the cylinder and is circulated by water that flows externally in a thermostatic bath to keep temperature of the solution constant at a definite value. Two thermocouples elements are inserted into the cell through waterproof openings on the bottom to monitor the solution temperature during measurements. A photographic view of the cell with the exploded components is reported in Fig.2

The filling procedure is such that solution is firstly inserted into the cell through the filling tube, while the membrane has still to be set at the top of the second tube and the central opening is open; then, with the second tube completely filled, the membrane is drawn and set in its final position with a small O-ring locking it to the tube and keeping it strained at the end of the tube. Finally, the cell is replenished through the central opening which is then closed with a proper lid on it.

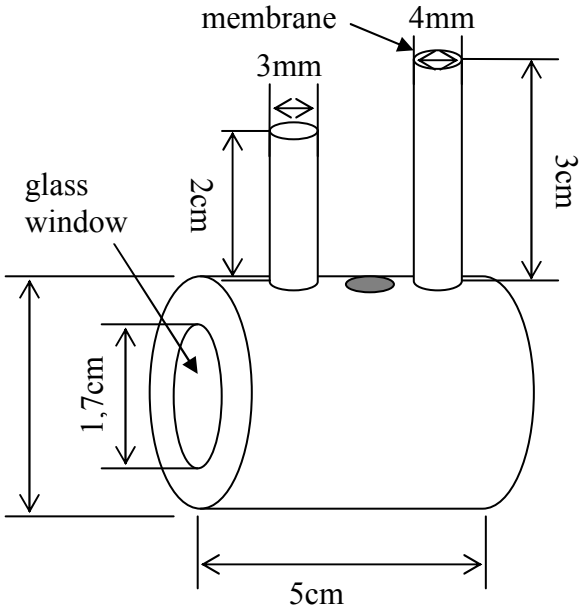

Fig.1 Schematic layout of the photoacoustic cell

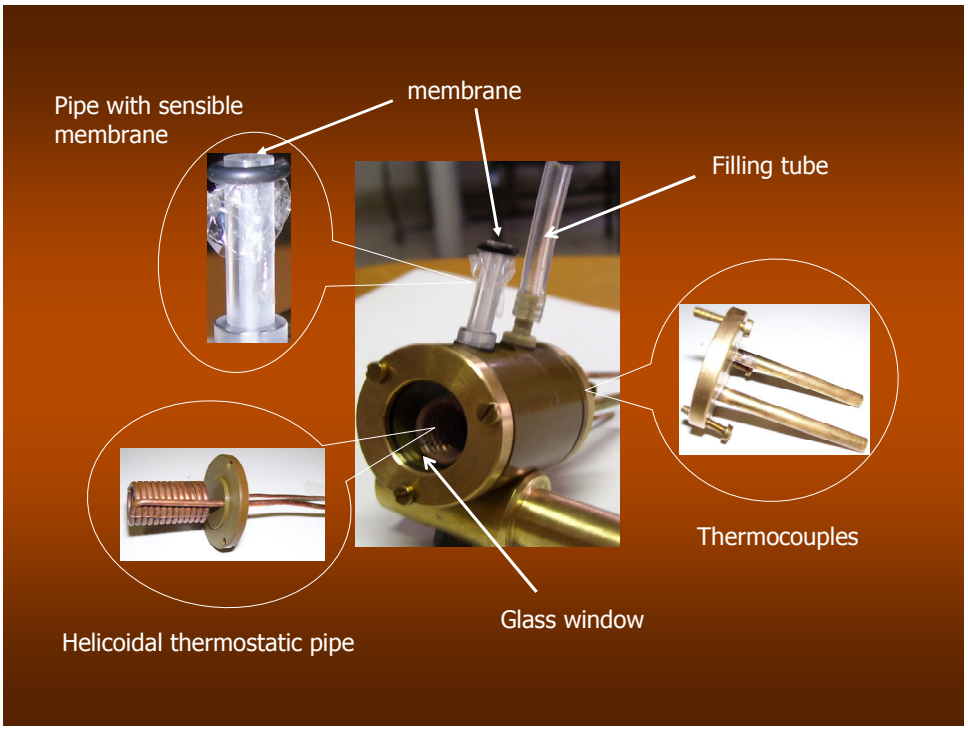

Fig.2 Exploded picture of the cell with the single components

3 Experimental set up

Photoacoustic detection of the solution volume variations is performed by illuminating the solution along the central axis of the cell with a variable cw power laser beam from an Argon ion laser, ranging from 0 to 100mW; the impinging light is chopped with a mechanical blade rotator at a variable frequency from 100 to 1000Hz and directly sent into the cell through the glass window (see Fig. 3). Electromagnetic power is released into the solution and heat causes the water volume to expand at the expenses of the movable membrane at the end of the pipe. Membrane vibrations are, then, monitored and measured by an acoustooptical probe through heterodyne processing of a laser beam reflected by the oscillating surface of the membrane. The output signal of the acoustooptical probe is then fed into a lock-amplifier, whose output is then recorded and properly processed for computational analysis.

The cell may be moved with respect to the acoustooptical probe in the plane of the membrane, so as to allow a scanning of its vibration amplitude, either along a line, or over all of it. This may correct any default introduced in stretching the membrane over the end of the pipe, or may be used to position the probing beam at the maximum vibrating point to follow the vibration amplitude vs power of the excitation light beam and to check the linearity of the photoacoustic response. Phase of the vibration could also be monitored and this may be of great help to correctly localize resonance conditions when changing the excitation frequency.

4 Results

Preliminary to measurements relative to solution characterization, a series of experiments has been performed with the cell filled with plane water. It is necessary, indeed, to know which is the frequency response of the cell structure when no contrast agent is present in the solution, wherefrom to deduce the variations induced by the added material.

A model is, therefore, introduced to justify the response of the water filled cell, as it is represented in Fig.4, where the two sections represent the amplitude and phase frequency response, respectively, as monitored by the membrane vibration, picked at the central point of it where oscillation is a maximum. A resonance of the structure is evident at frequency f=700Hz, where the amplitude is a maximum and phase changes by 180 degrees. Measurements have been performed both by increasing the frequency from the lowest to the highest value, and vice versa; in either case both the amplitude and the phase curves do match one the other, thus confirming the reliability of the results. The cell may be modeled as a Helmholtz resonator, as it is commonly known a bottle-like container in air, where the compliance of the filling air acts as a spring for the oscillating mass, represented by the air in the neck of the bottle. In our case, however, the filling air in the bottle is substituted by water, with a much higher compressibility factor (lower compliance) and density as well: proper frequencies do not greatly differ from those of air resonators. Resonance frequency for a cell with a single pipe alone, will be given by:

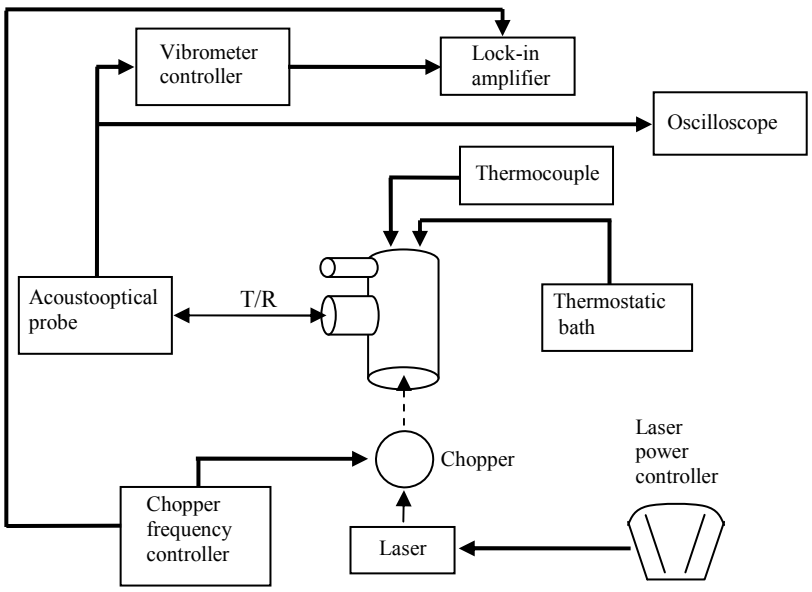

Fig.3 Block diagram of the experimental set up

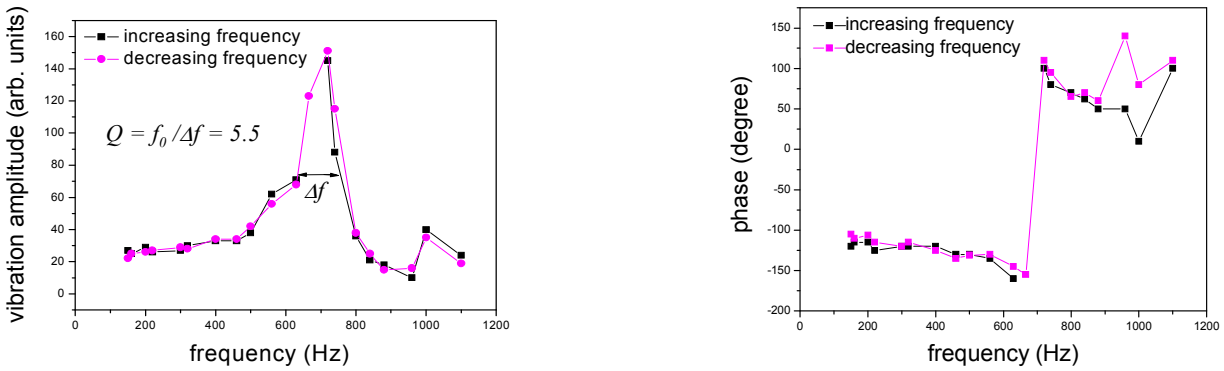

Fig.4 Amplitude (left) and phase (right) response of the photoacoustic cell vs frequency, filled with plane water

$$\omega = \sqrt{\frac{KA}{\rho l V}} \qquad (1)$$

where K=2,2 10^9Nm^{-2}, is the compressibility of water; A=0.5mm^2, is the pipe section area and l=25mm its length; V=7,7cm^3, is the cell internal volume and ρ=10^3kgm^{-3}, is the water density. These values will give a value of the frequency equal to f_o=$\omega/2\pi$=615Hz, which is really close to the value f=700Hz found experimentally. Improvement of the model would require the second pipe to be taken into account, which will introduce a second resonance frequency next to the one calculated, and the membrane compliance, whose proper frequency, however, would be low enough to be discarded in the range of frequencies presented. A correct evaluation of the proper modes of the cell will be presented elsewhere, while for the present case the preliminary approach presented is sufficient for interpreting the variations introduced by the microbubble contrast agent in the cell volume. The scanning of the membrane vibration amplitude is reported in Fig.5 (b-curve) at the mode resonant frequency f_o, along one diameter of the membrane, showing no nodes but at the ends of the segment; this is a demonstration that the oscillation of the cylinder mass in the pipe has no azimuthal nor radial modal numbers. Indeed, the acoustooptical scanning probe detects the velocity of the moving surface rather than its displacement; therefore, data should refer to velocity amplitude rather than to displacement, which, however, does not matter since arbitrary units are given for the values reported.

The contrast agent that has been experimented on is a microbubble solution SonoVue produced by Bracco SpA Milan, Italy. Microbubbles have an average diameter 2R=2.5µm, with 90% of them below 6 µm and 99% below 11 µm. They are filled with an inert gas of SF_6 and encapsulated with a thin membrane of phospholipids (Macrogol 4000, DSPC, DPPG, Palmitic acid).

Microbubbles are available in lyophilized samples and are produced by injecting 5ml of sodium chloride solution in a flacon containing 25mg of SonoVue powder. Solution is then active for an estimated time of 6 hours.

SonoVue has been introduced in the photoacoustic cell and the effect of it is clearly visible from the reduction of the photoacoustic signal detected by scanning the sensible membrane along one diameter of its.

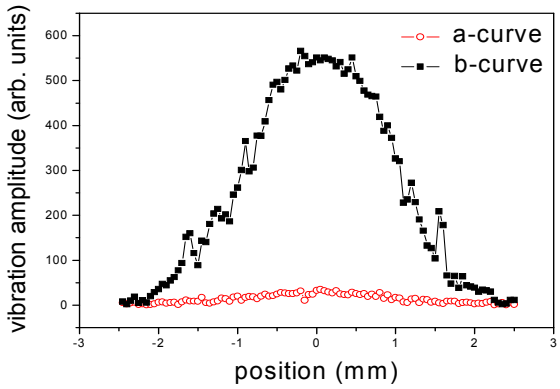

Fig.5 Comparison between vibration amplitude of the membrane, along one diameter, with plane water or contrast agent solution filled cell filled

Figure 5 is the velocity amplitude obtained by scanning one diameter, performed at temperature t=37°C, with (*a*-curve) and without (*b*-curve) the SonoVue solution: a drastic decrease is visible of the maximum amplitude at the central scanned line, where the value drops from 540 to about 35 and is due to the additional compliance of the solution microbubbles. The filling gas in the microbubbles, indeed, acts as a compensating chamber for the dilation of the water volume when it gets heated by the impinging radiation, thus reducing the vibration amplitude of the membrane which is a measure of the vibration amplitude of water in the cell pipe.

Temperature of the solution was, then, varied by changing the temperature of the thermostatic bath and three values have been experimented on, which are t=37 °C, 40 °C and 43°C. Obviously enough, time was waited for on moving from one set of measurements to the next one, in order to allow the thermal equilibrium conditions to set up and this may be accounted for to correctly interpret the results. In any case, however, a sensible variation was visible in the photoacoustical response when changing the thermal conditions.

Figure 6 represents the vibration amplitude of the membrane along one of its diameter, at the temperature values reported above. The amplitude sensibly increases when temperature is moved to 43°C, thus attesting that bubble conditions do change at that value, as if their membrane would be stiffened and compliance reduced. This result, however, could not be directly taken to deduce bubble compliance variation, since proper consideration should be paid to the time decay that bubble number and volume would certainly undergo in the lapse of time from one measurement to the next. Additional experiments should be done in order to evaluate the correct contribution to compliance variation of the solution due to the microbubble membrane variations.

Conclusions

A cell has been designed and realized for photoacoustical detection of signals from a SonoVue microbubble contrast agent. The cell is closed by a sensible membrane, set into

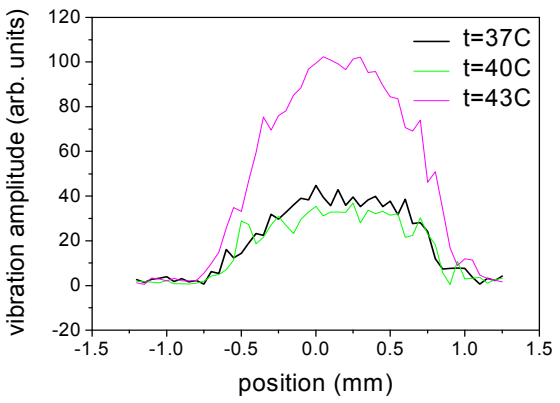

Fig.6 Vibration amplitude of the membrane, along one diameter, with plane water filled cell

vibration by the periodical volume oscillations of the solution filling the cell, thermally excited by chopped laser radiation. The cell is also thermostatically operated, so as to characterize the filling solution with respect to its temperature dependence. In order to do this, previous characterization has been performed with plane water filling cell and detection has been done both of the sensible membrane vibration amplitude and of the frequency dependence. Successively, measurements have been done with solution filling the cell and dreastic reduction of the signal has been found, due to variation of the solution compliance with respect to pure water. Temperature variation has been the final test on the cell performance, and results seem to be promising for testing contrast agent membrane vs temperature dependence.

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