ESTIMATION AND MEASUREMENTS OF RESONANCE SCATTERING ON THE GAS FILLED POLYMER MICROSPHERES

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The gas filled polymer spheres are used either as an ultrasonic contrast agents or controlled drug delivery microcapsules. The power spectrum of the ultrasonic backscattered signal was calculated from the resonance scattering theory for the gas bubbles surrounded by elastic shield. The size distribution of the measured microspheres was included in the calculations. In experiment, the backscattered power spectrum of measured sample was recorded by Siemens Antares ultrasonic scanner. Radio frequency (RF) data was recorded for 2.5 – 6.7 MHz transmitted ultrasonic frequencies. The backscattered spectra were calculated by Matlab software and subtracted from the transmitter spectrum, recorded as an echo from the perfect reflector. The particle size in measured sample was 12 µm mean ± 8 µm sd. The resonance frequency, measured under the microscope, was 0.60 MHz for 45 µm diameter microsphere which corresponds to 2.25 MHz for 12 µm sphere. The sample volume was 10cm³ and the mean quantity of scatterers was $6{\cdot}10^3$/cm³. In conclusion, measured spectra matched those calculated from theory. The use of ultrasonic scanner with RF data output and the high sensitivity, wide bandwidth ultrasonic transducer allows to measure backscattered signal from the very small quantity of resonance scatterers with satisfactory results at 40 dB signal to noise ratio.

INTRODUCTION

The gas filled lipid, protein or polymer microbubbles are used either as ultrasonic contrast agents [3] or the controlled drug delivery microcapsules [10]. The nonlinear behavior at resonance frequency and high backscattering cross section predisposes microspheres to use as a contrast particles for ultrasonic medical diagnostics. Combined with harmonic imaging [9], microbubbles are used for the blood flow studies and tissue perfusion imaging. In the other application, the microcapsules are filled with drug. High-pressure ultrasonic wave is used for the polymer shell destruction and the controlled drug delivery into the tissue or blood. This technique allows healing the restricted space area like a tumor. The size of the spheres varies between 2 - 6 μm for the contrast agent particles and between 1 – 50 μm for the drug delivery microcapsules. Microbubble destruction requires correct ultrasonic frequency equal to its resonance. This frequency depends on the bubble size and polymer shell stiffness. Measurements of the ultrasonic signal, backscattered from microspheres gives practical information of the bubble resonance and nonlinearity [5]. To obtain sufficient signal to noise ratio and wide bandwidth, either multiple transducers [1,4,6,8] or attenuation only was measured [12]. Authors propose the new technique of backscattered signal measurements, based on commercial wide bandwidth ultrasonic transducer and RF data recording.

1. THEORY

The scattering properties of the gas filled microspheres were derived from the resonance scattering theory. The total scattering cross section of a single bubble can be expressed as follows [11]:

$$\sigma_s = \frac{4\pi R^2}{\left(\frac{f_r^2}{f^2} - 1\right)^2 + \delta^2} \tag{1}$$

where R = bubble radius, f_r = bubble resonance frequency, f = frequency of the incident ultrasonic wave, δ = total damping constant caused by the surrounding liquid medium.
The resonance frequency of a gas bubble immersed in water is [3,7]:

$$f_r = \frac{1}{2\pi R}\sqrt{\frac{S_a}{m} \cdot b \cdot \beta} \tag{2}$$

where S_a = adiabatic stiffness of the gas, m = effective mass of the system, b = 1/A (A = polytropic coefficient), β = surface tension coefficient.
When a gas bubble is surrounded by a shell, the shell causes an additional restoring force. Then equation (2) is modified [2,3,7]:

$$f_{rs} = \sqrt{f_r^2 + \frac{S_{shell}}{4\pi^2 m}} \tag{3}$$

where f_{rs} = resonance frequency of the gas filled microcapsules with shell, S_{shell} = stiffness of the shell.

For the 12 μm diameter air bubble (without the shell), the resonance frequency is f_r = 0.54 MHz. The scattering cross section σ_s of single bubble, derived from the equation (1), is presented on Fig.1a.
If the shell mass $m_{shell} \gg m_{gas}$ then:

$$m = 4\pi R^2 h_{shell}\, \rho_{shell}$$

(4)

where h_{shell} = shell thickness, ρ_{shell} = shell density. Then the resonance frequency of the bubble with shell is:

$$f_{rs} = \frac{1}{2\pi R}\sqrt{\frac{S_a \cdot b \cdot \beta}{m} + \frac{S_{shell}}{4\pi\, h_{shell} \cdot \rho_{shell}}} \tag{5}$$

If the h_{shell} has the constant value, then equation (5) is modified:

$$f_{rs} = \frac{1}{2\pi R} \cdot A \tag{6}$$

where A = constant value.

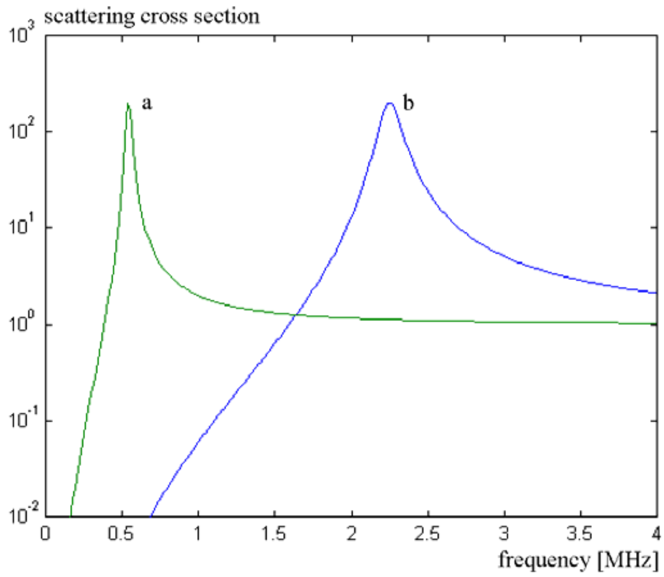

Fig.1. The scattering cross section σ_s of the 12 µm air bubble versus frequency. a. air bubble without the shell, resonance frequency was calculated from the equation (2), b. air bubble with polymer shell from the tested sample, resonance frequency was measured under the microscope

2. MATERIALS AND METHODS

The sample of air filled microspheres with polymer shell was used in the experiment. The mean diameter of the bubble was 12 µm ± 8 µm standard deviation. The size distribution measured by Coulter© cell counter is presented on Fig.2.

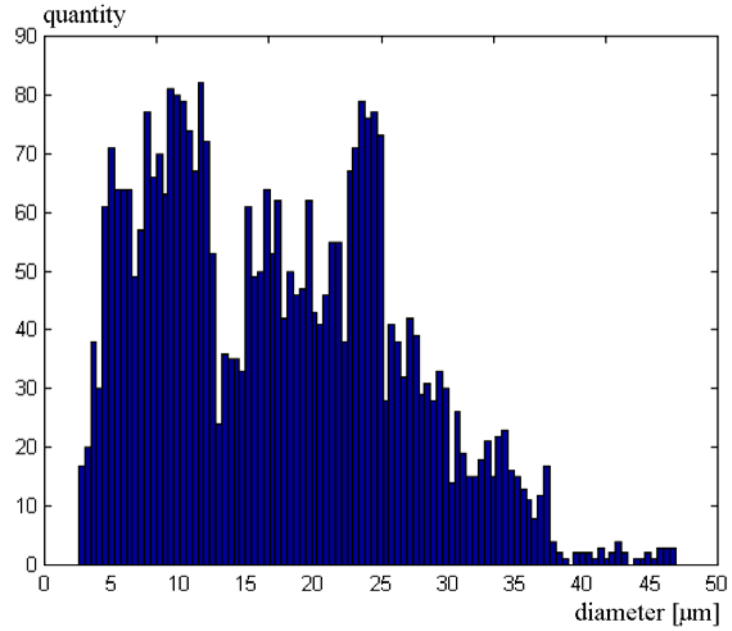

Fig.2. Histogram of the bubble size distribution of the sample used in experiment

The resonance frequency was measured using Nikon Eclipse-100 inverted microscope with Nikon LU Plan 10x/0.30 lens. The sample was illuminated by Schott KL-200 light source with goose neck fiber light guide. Microcapsules were suspended in 1 mm thick layer of agarose gel. The sample was sonified by custom made 50 mm diameter ultrasonic transducer focused at 75 mm. The -3 dB frequency range was 0.45 – 0.83 MHz, measured by hydrophone. Transducer was excited by 20 periods sine burst generated by Ritec RAM-10000 power generator. The maximum negative pressure was P_{-} = 3 MPa. The acoustical pressure was sufficient for destruct the microbubble at its resonance frequency. Diagram of the microscope setup is presented on Fig.3, and the microscope image of the destructed microcapsule is presented on Fig.4. Experiment was repeated to measure resonance frequency of a different size microbubbles.

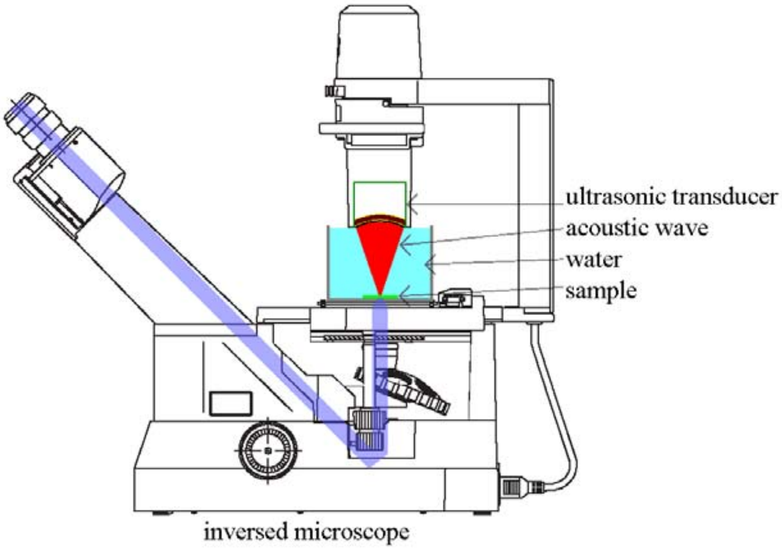

Fig.3. Microscope setup for the measurement of the microbubble resonance frequency

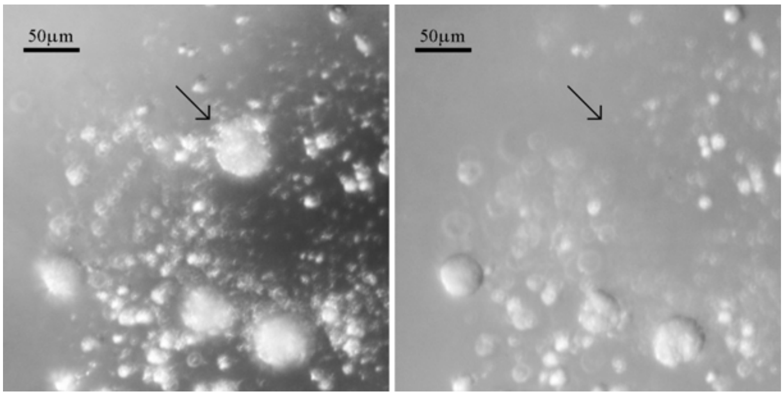

Fig.4. Microscope image of 45 μm microbubble with resonance frequency f_r = 0.60 MHz (left). Microbubble disappeared after the destruction caused by ultrasonic wave (right)

In the second experiment, the backscattered power of the measured sample was recorded by Siemens Antares ultrasonic scanner equipped with 2 - 6 MHz convex transducer. Microcapsules were suspended in 5 mm thick layer of agarose gel with 10 cm^3 total volume. The mean quantity of scatterers was $6{\cdot}10^3/cm^3$. Agarose sample was positioned between two 2 cm thick layers of the pure agarose. On the bottom was a layer of silicone to absorb ultrasonic waves and reject multiple reflections in the phantom. The sample was sonified from the top. The ultrasonic beam was focused at 4 cm, at the layer with microcapsules. The ultrasonic image of the measured sample is presented on Fig.5. The radio frequency (RF) data was recorded for 5 transmitted ultrasonic frequencies 2.50 – 6.67 MHz. 300 lines of RF signal were recorded at 40 MHz sampling frequency. Frequency spectra were calculated for each line and averaged. As a reference, ultrasonic signals reflected from the plastic ball were recorded for the same transmitter frequencies. The backscatterd spectra were calculated by the Matlab® software and subtracted from the transmitter spectrum, recorded as a reflection from the perfect reflector.

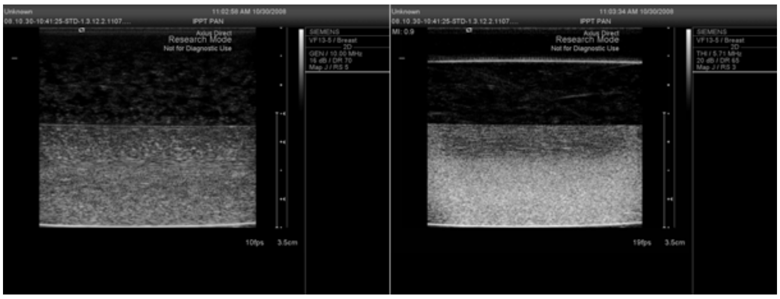

Fig.5. Ultrasonic image of the resonance scatterers suspended in the central layer of the agarose gel. Those particles generate strong ultrasonic echoes (bright area) at 5.7 MHz harmonic imaging (right) compared to low echo intensity at 10 MHz image (left)

3. RESULTS

The resonance frequency of a polymer coated gas bubble from the measured sample and calculated from the equation (6) is:

$$f_{rs} = \frac{13.5}{R} \tag{7}$$

where f_{rs} = resonance frequency in MHz and R = bubble radius in µm. For the 12 µm diameter microcapsule (mean value in sample), the resonance frequency was 2.25 MHz.

The backscattered power was estimated as a sum of scattered cross sections of the microbubbles used in experiment. Based on equation (1), measured histogram of the size distribution and resonance frequency measured under the microscope, the frequency spectra of backscattered signal was calculated for 6000 scatterers. The scattering cross section versus frequency for 12 µm microbubble with polymer shell is presented on Fig.1b. The calculated frequency spectrum of backscattered signal for measured sample is presented on Fig.6.

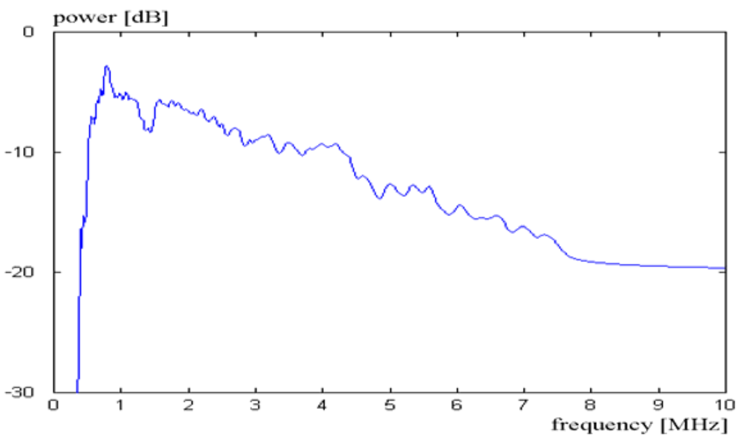

Fig.6. Calculated backscattered frequency spectrum of the measured microbubbles sample with polymer shell

The measured frequency spectra, backscattered on the measured sample are presented on Fig.7. The signal to noise ratio of the recorded signal was S/N ≥ 40 dB. And the frequency spectrum of the agarose sample of microbubbles with polymer shell, corrected by reference spectrum is presented on Fig.8.

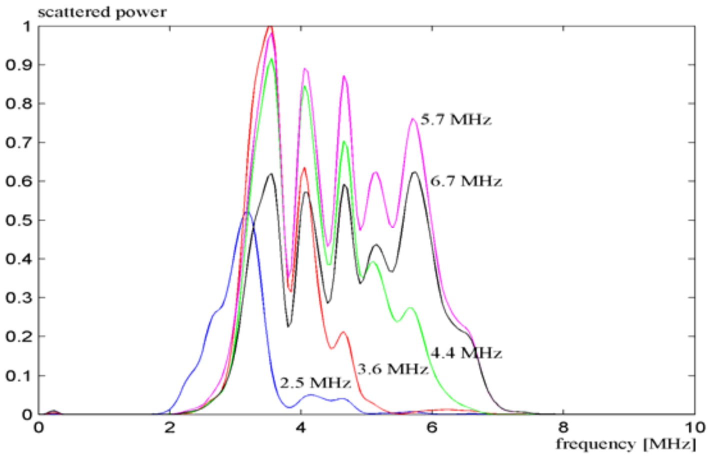

Fig.7. Measured frequency spectra, backscattered from the agarose sample of the microbubbles with polymer shell. Measurements were performed for the five transmitter frequencies 2.50 - 6.67 MHz

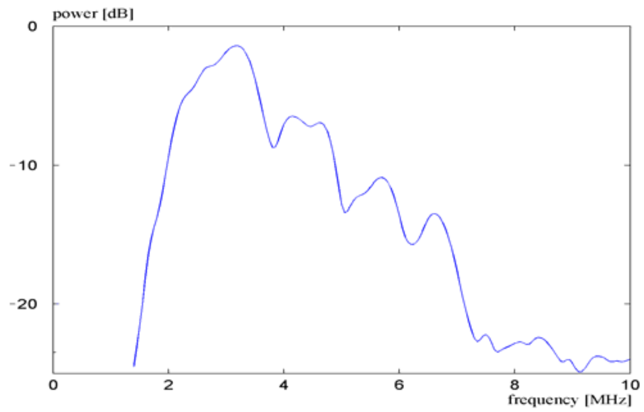

Fig.8. Measured frequency spectrum of the agarose sample of microbubbles with polymer shell, recorded at 2.50 MHz transmitter frequency and corrected by reference spectrum

4. CONCLUSIONS

1. The technique proposed by authors allowed to measure power of ultrasonic signal backscattered on polymer shell microcapsules in low concentration $6 \cdot 10^3$ particles/cm^3. In 2 - 6 MHz frequency range, the signal to noise ratio was S/N ≥ 40 dB. Those results were obtained using single probe and direct measurements of ultrasonic scattering signal, which is an improvement compared to the multi transducer ant attenuation only measurements [1,4,6,8,12].

2. The measured frequency spectrum of the backscattered signal matches those calculated from the resonance scattering theory based on equation (1) and measured particle resonance frequency given by equation (7) and the size distribution of measured scatterers (Fig.2). The microsphere resonance frequency, measured under the microscope, was 0.60 MHz for 45 μm diameter which corresponds to 2.25 MHz for 12 μm sphere.

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