

Title:

Characterization of individual submicron perfluorocarbon gas bubbles by ultrasonic backscatter.

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Abstract

Measurements were undertaken to determine the unknown microbubble size distribution of a dodecafluoropentane (DDFP) emulsion consisting of 10^{12} droplets/mL in surfactant-stabilized water. The acoustic backscatter of 2-microsecond-duration tonebursts of 30 MHz ultrasound was measured from the emulsion as it moved in a coaxial jet flow. Calibration of the backscatter coefficients for the system was accomplished using 3 μm -radius polystyrene spheres, whose acoustic properties were assumed known. Applying viscous linear scattering theory allowed inversion of the backscatter data to find a mean microbubble radius of 130 nm.

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Introduction

Determining the size distribution of preparations of microbubbles is an ongoing challenge in fields as diverse as oceanography and biomedical ultrasound. Various methods have been employed to interrogate large populations of microbubbles and yield ensemble-averaged parameters that, in turn, provide information about bubble size distributions. All acoustic ensemble techniques assume that the greatest contribution arises from bubbles near resonant size, although Commander and Prosperetti (1989) noted the important contribution of off-resonance bubbles to measured spectra. In addition, bubble ensembles may possess low-frequency collective resonances due to bubble-bubble coupling that incorrectly imply much larger bubbles than those actually present (Nicholas et al. 1994).

Methods that interrogate a single bubble at a time avoid these pitfalls. Techniques include frequency-dependent backscatter (Leighton 1994, Kapodistrias and Dahl 2002), optical laser scattering (Gaitan and Holt 1999; Arnott and Marsten 1988; Weninger et al. 1997), white light photography (Porter et al. 1996; Dayton et al. 1999), Coulter-counters (Shi et al. 1999), and hybrid acoustic-optical methods (McCormick 2002). For the acoustical methods, ambiguities in size determination occur based upon whether a bubble is larger or smaller than the resonance radius size. Inversion of backscatter data requires *a priori* knowledge of the monotonic relationship between bubble size and scattering function for the range of radii interrogated. For optical methods, technological barriers include low sensitivity for bubbles smaller than one micron radius and the necessity for transparent host liquids.

Of great interest in biomedicine is the characterization of echocontrast agents used in biomedical ultrasound applications such as cardiology. Bubbles typically are manufactured in a size range smaller than 3- μm radius, with number densities above 4×10^8 bubbles/mL (Bleeker and Shung 1990), and may contain air or perfluorocarbon gases of low solubility in blood. Moreover, some echocontrast agents have surface coatings of lipid, surfactant, or protein that affect the bubble dynamics and hence the inferred bubble size based upon acoustic interactions (Church 1995, Khismatullin and Nadim 2002).

Of interest here are emulsions of dodecafluoropentane (DDFP) droplets used in echocontrast agents such as Echogen TM (Correas and Quay 1996). Since the boiling temperature for this material is approximately 28-30 °C at one atmosphere pressure, individual droplets will be superheated at body temperature (37 °C). If a nucleation mechanism allows some droplets to boil and hence become bubbles, the low solubility of DDFP will allow the bubbles to persist in the circulatory system and provide sustained ultrasonic contrast for the vessels or tissues in which they are present.

Theory

The objective is to determine a bubble's size given knowledge of its acoustic backscatter strength at a single frequency. Inversion of the measurement requires a forward model that predicts the backscattered pressure field as a function of known physical parameters.

We consider monochromatic plane waves incident upon an inviscid fluid/elastic sphere of radius a suspended in a fluid host, where both the sphere and the host materials are compressible and

non-conducting, and the boundary conditions are the continuity of normal velocity and stress across the surface of the sphere. In this case, the far field scattered pressure amplitude $p_s(r)$ can be expressed in the following form (Hay and Burling, 1982):

$$p_s(r) = \left[\frac{P_0}{r} \exp(ikr) \right] \Phi, \quad (1)$$

where r is the distance from the center of the scatterer to the field point, P_0 is the amplitude of the incident plane wave, k is the wavenumber in the host fluid, Φ is the angular distribution function that modulates an outgoing spherical wave in the far field and is given by

$$\Phi = \frac{i}{k} \sum_{m=0}^{\infty} (2m+1) \sin \eta_m \exp(-i\eta_m) P_m(\cos \theta), \quad (2)$$

In Eq. 2, P_m is the Legendre polynomial of order m and argument $\cos \theta$; θ is the scattering angle referenced to the forward direction ($\theta = 180^\circ$ for backscattering) and η_m is the phase angle of the m^{th} partial wave. The phase angle results from the imposition of the boundary conditions.

The function Φ contains all the information regarding the size, dynamic response, and acoustic contrast of the scatterer. In the particular case of a fluid sphere, it can be expressed as follows

$$\Phi = \frac{1}{k} \sum_{m=0}^{\infty} \frac{(2m+1)}{1+iC_m} P_m(\cos \theta), \quad (3)$$

where

$$C_m = -\cot \eta_m = \frac{-\frac{dy_m(x)}{dx} + \frac{y_m(x)}{j_m(x')} \frac{dj_m(x')}{dx'} \frac{\rho c}{\rho' c'}}{-\frac{dj_m(x)}{dx} + \frac{j_m(x)}{j_m(x')} \frac{dj_m(x')}{dx'} \frac{\rho c}{\rho' c'}}, \quad x = ka, \quad x' = k'a, \quad (4)$$

ρ and c are the density of and sound speed in the host fluid, and $j_m(x)$ and $y_m(x)$ are spherical Bessel functions of the first and second kind, respectively. A prime denotes the parameters of the fluid sphere.

It is important to keep in mind that the DDFP bubbles considered in this work are less than 1 μm in radius. The dynamics of their oscillation, and hence acoustic response, should be dominated by the viscosity of the host fluid, μ . As shown by Khismatullin (2004) and Khismatullin and Nadim (2002), viscosity shifts the resonance frequency of the backscattered signal to a higher value and even eliminates completely the resonance peaks in the output-level versus frequency curves for bubbles smaller than the critical size. The following assumptions are made to include host fluid viscosity in the angular distribution function. First, viscosity is taken into account only in the monopole mode ($m=0$). Because the bubble's size is much less than the wavelength in the host fluid ($x \approx 0.0126$ for DDFP bubbles of radius 100 nm in water at 30 MHz), the monopole mode is the greatest contribution to the scattering strength. The Keller-Miksis equation (Keller and Miksis, 1980) [see also Eq. (64) in Khismatullin and Nadim (2002)] is used to describe these oscillations. Second, thermal damping is disregarded because it is two orders of magnitude less than viscous damping (Khismatullin and Nadim, 2002). Third, the monopole term of the scattered pressure field is linear with respect to bubble radius perturbation. This assumption is valid because the acoustic pressure amplitude is much less than the atmospheric pressure in this work. Linearization of the equation for far-field scattered pressure [Eq. (11) in Khismatullin (2004)] leads, at $ka \ll 1$, to the following viscous correction to the coefficient C_0 :

$$i \frac{4\mu}{\rho c a x^2}. \quad (5)$$

Thus the forward scattering model consists of gaseous DDFP suspended in water. The density and sound speed for the DDFP gas is taken to be 11.391 kg/m³ and 93 m/s (Flaherty, personal

communication), respectively, and the host fluid is assumed to be pure water with viscosity $1 \text{ cP} = 0.001 \text{ Pa} \cdot \text{s}$ at 37°C .

Apparatus and Methods

Individual bubbles were convected through the focus of a 30 MHz acoustic transducer, driven in pulse-echo mode, using an apparatus based on that of Roy and Apfel (1990). The acoustic beam axis was oriented horizontally and the trajectory of the flow was in the focal plane of the transducer and perpendicular to the beam axis. A 3.2 mm-diameter brass ball was used as a scattering target for alignment of the ultrasound beam in a 21.6 cm (w) by 15.24 cm (h) by 29.1 cm (l) acrylic tank filled with clean distilled water at 37°C . 30 MHz echoes that maximized specular reflection from the ball were used to locate the position of the transducer focus and served as a calibration reference. Two measuring microscopes positioned in the horizontal plane (perpendicular and parallel, respectively, to the acoustic axis) were used to record the position of the focus. Later, a coaxial jet flow consisting of an outer sheath flow and inner plug flow was positioned to pass through the acoustic focus, based upon the microscope position (the jet was visualized by seeding the inner flow with India ink and proper alignment was confirmed by observing increased backscatter from the jet when ink was present). The outer sheath flow consisted of distilled water from a gravity feed at a continuous rate of 225 ml/h, while the inner plug flow was degassed Ringer's solution driven by a syringe pump at a constant flow rate of 1.4 ml/h. The inner flow was introduced coaxially into the outer flow via a 30-gauge needle located upstream of a narrowing in a glass capillary tube. This tube's inner diameter at the jet outlet was approximately 200 μm , with inner flow diameter approximately 10 μm . The inner flow moved at nearly constant velocity (plug flow) vertically downward and into a suction drain (siphon) located approximately 1 cm below the acoustic focus.

A 0.2- μm Nalgene filter was used at the syringe outlet to minimize particulate contamination of the inner flow. India ink, polystyrene spheres in suspension, or DDFP emulsion was infused at a known rate from a computer-controlled syringe pump (Cole-Parmer U-74900-10, Vernon Hills, IL) to help visualize the inner plug flow (ink), provide calibrated scatterers (polystyrene spheres), or supply unknown scatterers (DDFP emulsion). Employing separate ports for each of these “seed” flows into the inner flow helped avoid cross-contamination.

The unbacked, single-element ultrasound transducer was driven in pulse-echo mode with a 2- μs -duration, 30 MHz toneburst of 40 V peak-to-peak amplitude at a pulse repetition frequency (PRF) of 4 kHz. Gating and filtering electronics allowed the returned echoes to be displayed on a Tektronix 2455B analog oscilloscope. A detailed description of the signal generation and primary detection apparatus is provided in Roy and Apfel (1990). A monitor output of the oscilloscope yields an exact duplicate of the displayed signal, scaled in amplitude so that a full scale signal on the screen (10 divisions) corresponds to 1 volt at the monitor output. This voltage was input into a Panametrics Model 5607 gated peak detector (GPD; modest amplification was employed on order to fully utilize the dynamic range of the digital peak detector). The DC output of the GPD was recorded synchronously by a computer using a 12-bit analog-to-digital converter card (National Instruments). Reflections from the brass ball target were used to measure the linear transfer function relating the peak echo voltage to the DC output of the GPD. This relationship was used to convert the recorded GPD output voltage to an echo voltage amplitude during post-processing.

Calibration, Data Processing, and Inversion

Upon establishing a stable coaxial jet flow, calibration data were obtained by infusing polystyrene spheres (3.019 μm mean radius; Duke Scientific Company) in the inner plug flow via an injection port. Scattering events were recorded for 60 s. Figure 1 shows a segment of raw data from the output of the GPD for the 6- μm -diameter polystyrene spheres. Each circle represents the amplitude of an ultrasonic pulse reflected from a polystyrene sphere as the scatterer was convected through the focus. The data takes on the form of discrete peaks for, at a PRF of 4 kHz, a target is interrogated multiple times as it traverses the focus; the peak echo voltage generated by the passage of a given sphere determines the scattering strength for that sphere. However, at high concentrations, there is a risk of having more than one sphere in the focus at the same time. A peak-finding routine was used to identify each point for which the second derivative of the function was negative (concave downward) and for which at least the four points preceding it produced positive function slope and at least the four points following it produced negative function slope. Such points are represented in Figure 1 by red plus signs. This procedure served to reject multiple peaks associated with the near-simultaneous passage of multiple scatterers (“coincidence scattering”) and also to reject small peaks resulting from noise. Since the jet velocity and PRF were nearly constant (independent of particle size), the peak width represents the transit time through the acoustic focus of an individual particle.

Mm. 1. Fig1.mpg (889 kb). This is a file of type “mpg” and should be available to authorized reviewers and editors of ARLO.

The next step in the processing scheme was to estimate the maximum echo voltage for a given peak in the data stream, given that the particle is sampled 8-9 times as it traverses the main lobe of the ultrasound beam, which we approximate as Gaussian. For each such candidate peak, a 2-parameter nonlinear least-squares fit of the Gaussian function $y = A\exp(\frac{-x^2}{\sigma})$ was made to the nine points defining the peak. Variations in the amplitude parameter A and the width parameter σ for successive peaks arise from variations in particle size and liquid jet trajectory through the acoustic focus, respectively. From the mean polystyrene sphere radius supplied by the manufacturer, the system sensitivity parameters Φ and P_0 (Eq. 1) may be calculated.

Knowledge of transmitted and backscattered signal voltages, from which the system sensitivity parameters Φ and P_0 are derived, can be used to determine the linear scattering function of fluid scatterers (bubbles). For each unknown scatterer, the width parameter σ provides an estimate of transit time through the acoustic focus, and can be used to discard non-compact scatterers such as DDFP filaments, “pearl chains,” or other coincidence scattering events. For DDFP backscattered data, all candidate peaks were discarded whose width parameters σ were larger than twice the standard deviation in σ for the polystyrene sphere data. Although there still may be some coincidence scattering present in the remaining data, the result is a potential overestimation of the bubble size, based upon the larger scattering cross-section of multiple bubbles present in the acoustic focus as opposed to that of a single bubble. Thus the remaining peaks were assumed to be individual microbubbles of DDFP, and their amplitude parameters A were used with viscous linear scattering theory to determine a conservative estimate of their individual radii.

A DDFP emulsion was infused into the inner plug flow at a rate of 1 microliter per hour at 37 °C. Prior to infusion, the bottle containing the emulsion was refrigerated at 8 °C for 2 h and then vented with an 18-gauge needle. Another 18-gauge needle was used to draw the chilled emulsion into a 30 cc polyacrylate syringe, which was stored upright for 0.5 h. All foam and large bubbles were ejected from the syringe, and it was inverted and held at 10 °C in a computer-controlled syringe pump (Cole-Parmer 74900) located inside a refrigerated Styrofoam box. The infusion rate of 1 μ l per hour is the lowest infusion rate obtainable with the syringe pump, and results in an approximately 1000-to-1 dilution of the emulsion into the inner plug flow. When the pump was inactivated, the backscatter quickly returned to a constant level below 0.2 divisions.

Results

The center panel of Figure 2 shows the radii inferred from the amplitude parameter A for each of four hundred 6- μ m-diameter polystyrene spheres that have passed through the acoustic focus. The uppermost panel of Figure 2 shows a linear elastic scattering theory curve, on which are plotted the radii of individual spheres as the result of inversion using the properties for polystyrene (Roy and Apfel, 1990): 1110 m/s shear wave speed (elastic scatterer), 2380 m/s compressional wave speed, 1049 kg/m³ density at 37 °C . In the lowest panel of Figure 2, the distribution of polystyrene sphere sizes is centered at radius 3.019 μ m and serves as the calibration data with reference to which the DDFP data are compared.

Figure 3 shows the raw data stream of backscatter echo amplitudes from diluted DDFP emulsion as it passed through the acoustic focus. Candidate peaks are marked with a red plus sign. All peaks with width parameters σ larger than two standard deviations of the width parameters for the polystyrene spheres were rejected as caused by non-compact scatterers. Remaining peaks, presumably single DDFP bubbles, are marked with an asterisk above the peak.

Mm 3. Fig3.mpg (1096 kb). This is a file of type “mpg” and should be available to authorized reviewers and editors of ARLO.

The center panel of Figure 4 shows the individual bubble radii inferred from the amplitude parameters of the remaining 2295 DDFP bubbles passing through the acoustic focus. The uppermost panel of Figure 4 shows viscous linear scattering theory for DDFP bubbles below the critical radius, with monopole bubble resonance suppressed due to host fluid viscosity. The lowermost panel shows the result of inversion of the scattering data using the system sensitivity parameters obtained from the polystyrene spheres, as well as assumed parameters of DDPF gas. Thus the mean DDFP microbubble radius is calculated to be about 130 nm with a standard deviation of about 50 nm.

Summary and Conclusions

The DDFP microbubbles measured in this study were sufficiently small that viscosity of the surrounding fluid dominates their dynamics, leading to the removal of the resonance peak that inviscid theory would predict. Without a dominant resonance peak, inversion of the scattering data could be accomplished allowing calculation of a size distribution for the DDFP bubbles

between 100 and 200 nm radius, with diminishing numbers up to 600 nm. The size distribution is a conservative estimate (upper bound) since coincidence scattering (multiple bubbles in the focal region simultaneously) may have sometimes occurred. [Work supported in part by Sonus Pharmaceuticals and by The Center for Subsurface Sensing and Imaging Systems, under the Engineering Research Centers Program of the National Science Foundation (award number EEC-9986821).]

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Figure Captions

Figure 1 (refers to Mm1): Segment of output of Gated Peak Detector (V) for 6- μm -diameter polystyrene spheres. Horizontal axis is time or pulse number. Circles represent instantaneous Peak Detector values, while red plus signs denote peaks found that have four points of consistent slope on either side of the maximum.

Figure 2: Output of scattering inversion for 6- μm -diameter polystyrene spheres, using data for polystyrene (Roy and Apfel 1990). Uppermost plot shows scattering cross-section curve and where these data fall upon it; middle plot shows inferred radius based upon inversion; lower plot shows probability histogram of microsphere radius, with mean value 3.019 μm as per manufacturer.

Figure 3 (refers to Mm3): Segment of output voltage of Peak Detector as a function of echo number for the DDFP emulsion at 37 °C. Note that, in comparison to Fig. 1, some peaks are broader, while others are the same width as those obtained from 6- μm -diameter polystyrene spheres. Since the jet flow rate was the same as for the polystyrene spheres, broader peaks indicate scatterers that are not compact with respect to the width of the acoustic focus (270 μm). Compact scatterer peaks are those whose widths are no more than twice the mean of polystyrene sphere widths, and are marked above with an asterisk.

Figure 4: Result of inversion using the compact scatterer data (asterisked) of Fig. 3 and the properties of polystyrene spheres (Figure 2) for DDFP emulsion at 37 °C . Uppermost scattering

theory curve shows no resonance peak due to viscosity of surrounding fluid for bubbles smaller than the critical radius (Khismatullin 2004). Mean bubble radius is $0.130\text{ }\mu\text{m}$.







