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Quantitation of Echo-Contrast Effects

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ABSTRACT With the recent development of sonicated microbubble echocardiographic contrast agents, it is now reasonable to attempt to quantitate actual tissue perfusion. However, this requires an understanding of the quantitative relationship between microbubble concentrations and the reflected ultrasound signal. This paper describes (1) the basic acoustic properties of sonicated microbubbles, and (2) experimental verification of this relationship, showing that the ultrasound signal actually begins to decrease at a critical concentration that may be predicted based upon bubble size.

These microbubbles have acoustic properties that are essentially those of a random collection of Rayleighian scatters. Signal strength is governed primarily by the compressibility of gas, as opposed to fluid. In addition, bubble diameter is an important factor in determining signal strength (sixth power dependence). And, because the bubbles are randomly arranged, the reflected signal is not as great as might be expected, when compared to the signal reflected by a single bubble.

A simple in vitro test of the acoustic analysis confirmed the critical limit for bubble concentration, the measurement of which led to a prediction for sonicated microbubble size that is within a factor of two of the published values.

This acoustic analysis and confirmation, along with ongoing in vivo experimentation, promises to make possible quantitative regional perfusion measurement employing sonicated contrast agents.

Key words: Ultrasound, contrast, sonication, acoustics, perfusion

INTRODUCTION

Sonicated microbubbles used as echocardiographic contrast agents promise to make possible the quantitation of regional tissue perfusion. The sonicated agents could do this by serving as the "dye" for dye dilution measurements of blood flow. An ultrasound scanner then would make the "measurements" quickly throughout an entire view (tomographic slice) of an organ, allowing, for example, an echocardiogram to show areas of hypoperfusion at risk for infarction or a renal sonogram to show redistribution of flow in response to antihypertensives. This paper presents the basic acoustic analysis for quantitating echo contrast effects and in vitro verification.

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BACKGROUND

Sonicated echo-contrast agents are red-blood-cell-sized microbubbles suspended in a carrier solution. The early work on echo-contrast agents by Gramiak and Shah [1] focused on qualitative observations of blood flow using much larger microbubbles, up to 50 and 100 μm in diameter, produced by hand agitation or injection turbulence. Larger microbubbles are quite appropriate for observing valvular and vascular abnormalities [2,3], but become trapped in capillary circulation. Sonicated microbubbles are smaller (less than 10 μm) and more uniform in size. This allows them to flow unhindered through the capillary circulation [4,5].

Despite their size, hand-agitated contrast agents and other larger microbubble-based agents have been used to investigate coronary blood flow in animals [6–12] and humans [13,14]. This work has demonstrated that echo agents do indeed delineate areas of infarction. Furthermore, this work indicates that these larger microbubble agents are relatively safe even though they interfere with capiliary circulation.

Some quantitative work has been done on the size of the defects indicated, but not on blood flow.

Work with sonicated agents in humans [15] also has shown that the agents are safe and they generate the expected basic coronary perfusion patterns. A semiquantitative analysis of renal perfusion that uses sonicated agents has been undertaken [16], based on an analysis of videodensity curves from videotapes of two-dimensional renal sonograms performed simultaneously with contrast injections. The videodensity analysis confirmed the expected changes in the renal perfusion induced by various pharmacologic agents. This analysis was further verified by electromagnetic flow monitoring.

To summarize, microbubble echo-contrast agents are an effective and relatively safe method of characterizing blood flow. Now, with the development of sonicated microbubbles the size of red blood cells, it is reasonable to attempt to quantitate actual tissue perfusion.

ACOUSTIC ANALYSIS

For the purpose of this paper, the acoustics of sonicated echo-contrast agents are the acoustics of small gas bubbles. A small gas bubble in water is a very effective acoustic reflector [17]. Though air is orders of magnitude less dense than water, it is the incompressible nature of water relative to air that dominates. Both differences are additive and effect a large impedance discontinuity. This follows from the fundamental formulas for ultrasound wave reflection by a sphere developed by Rayleigh [18].

Figure 1 shows an ultrasound examination of the heart as a typical clinical contrast study. Figure 2 shows the same situation, but focusing down on a particular target volume and acoustic path of interest. The acoustic elements along the path have been simplified into transmission and reception geometry factors summarizing the performance of the transducer, a path attenuation factor taking into consideration the proximal anatomy not imaged (a squared factor as it is traversed to and fro), and finally the backscatter ratio for the small volume of interest. Separating the transmission and reception geometry factors allows proper treatment of linear, phased arrays.

The analysis in Figure 2 applies reasonably well only if the structures are stationary. For renal or hepatic studies this is generally true. For cardiac studies, it applies only during the transmission of a single pulse of ultrasound. Even then, it ignores Doppler effects and presumes ECG gating such that comparisons may be made from the same point in the cardiac cycle.

Now consider the effect of introducing contrast into the target volume. For the simplest analysis of microbubble contrast agents, treat each bubble as a small mirror of scattering cross-section Σ_s. A small volume filled with such an agent is depicted in Figure 3. The backscattered intensity is proportional to bubble concentration and scattering cross-section (Σ_s) considering only a small volume.

Reviewing the actual clinical situation in an echo-contrast study, it becomes clear that the contrast agent will occupy more than just a small volume. Typically, the contrast effect will fill the entire myocardium or renal parenchyma. Reviewing again Figure 3 note that the incident intensity is

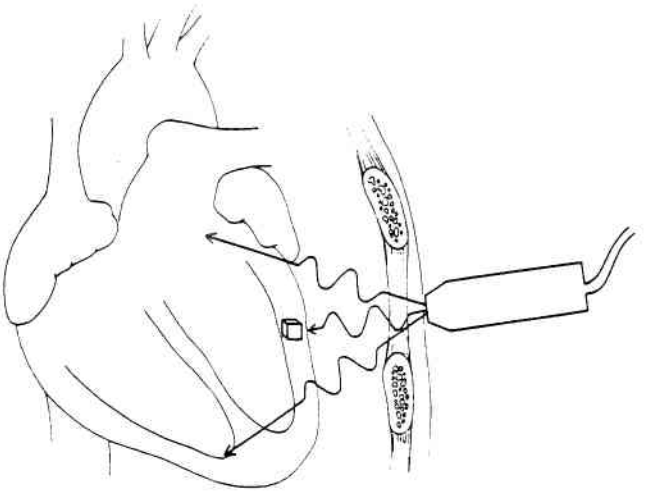
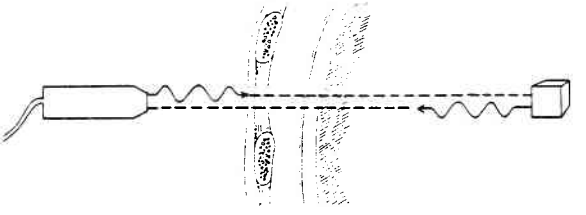


Fig. 1. Basic positioning of an ultrasound scanner's transducer (on right) to examine the human heart. Small cube represents a volume of specific interest.



Power-Received = Power-Transmitted x Transmission-Geometry-Fact
x Path-Attenuation-Factor^2
x Volume-Backscatter-Ratio
x Reception-Geometry-Factor

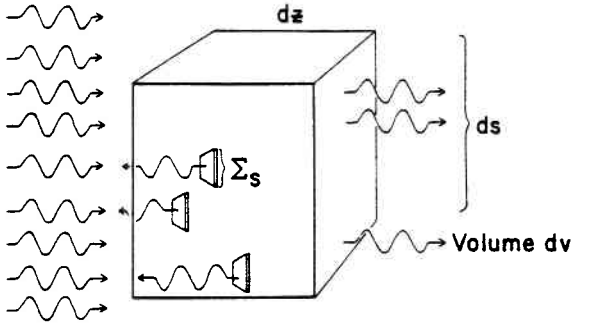
Fig. 2. Schematic depiction of acoustic factors for situation pictured in Figure 1.

diminished proportionally as it passes through each successive small volume. Its magnitude must be described by an exponentially decaying function along the path perfused with contrast agent. A more exact formulation is presented in Figure 4. The path factors unaffected by contrast agent injection are lumped together as "P-A-F^2" (path-attenuation-factor). Relative intensity can then be expressed simply in terms of bubble concentration N and two constant parameters K_1 and K_2 determined by the distance into the contrast volume, bubble size, fixed path factors, etc.:

$$I_R/I_T = NK_1e^{-NK_2}. \quad (1)$$

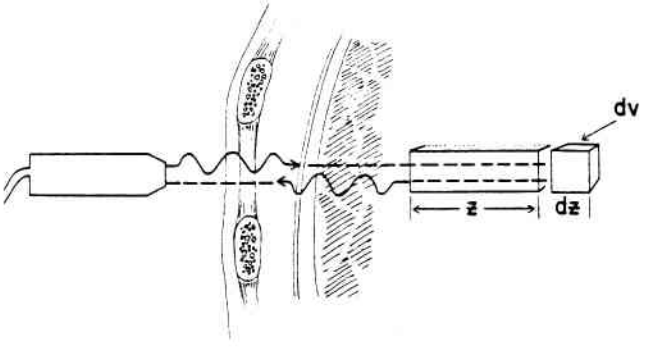
Note that the relative intensity is not simply proportional to (N) the bubble (and thus contrast agent) concentration. Indeed, above a certain concentration the intensity will actually decrease. This has been observed by the authors in the course of in vitro experiments.

Returning to an individual bubble, we use the formulations of Morse and Ingard [19]. Figure 5 depicts a plane wave coming from the left impinging on a small bubble in the center. The formula for the relative intensity of the reflected wave (assuming that the bubble radius is much less than the wavelength) is also shown. For a microbubble of air in



$$\frac{\text{Intensity - scattered}}{\text{Intensity - incident}} = \text{Number - scatters - per unit volume} \times \Sigma_s[\text{scattering cross section}] \times dz$$

Fig. 3. Ultrasound scattering by microbubbles (treated as a little mirrors in a small volume of interest, dv).



$$\frac{I_R}{I_T} = P\text{-}A\text{-}F^2 \times \left(e^{-2N\Sigma_s z}\right) \times \left(N\Sigma_s dz\right) = NK_1 e^{-NK_2}$$

Fig. 4. Relation of received ultrasound intensity (I_R) to transmitted intensity (I_T) as a function of path attenuation factor (P-A-F), microbubble concentration (N), cross-sectional area (Σ_s), and acoustic path through contrast agent.

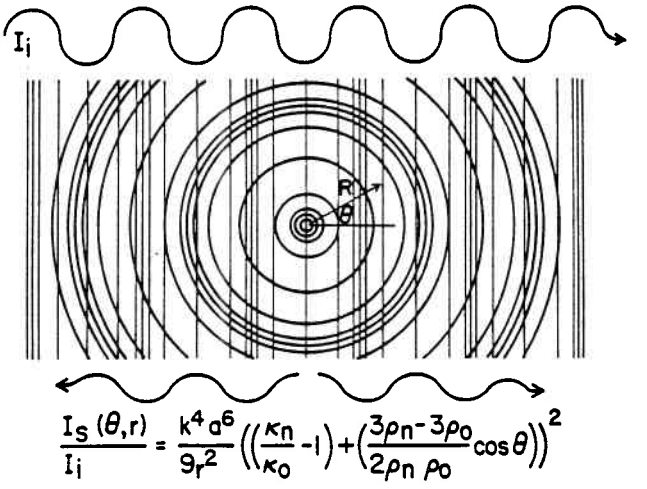


Fig. 5. Rayleigh scattering by a microbubble (center) of a sound wave intensity I_i (from left) resulting in a scattered wave (I_s). r,θ are coordinates relative to bubble, k is wave number, a is bubble radius, κ is compressability, ρ is density, subscript $_0$ is for contrast agent, subscript $_n$ is for bubble gas.

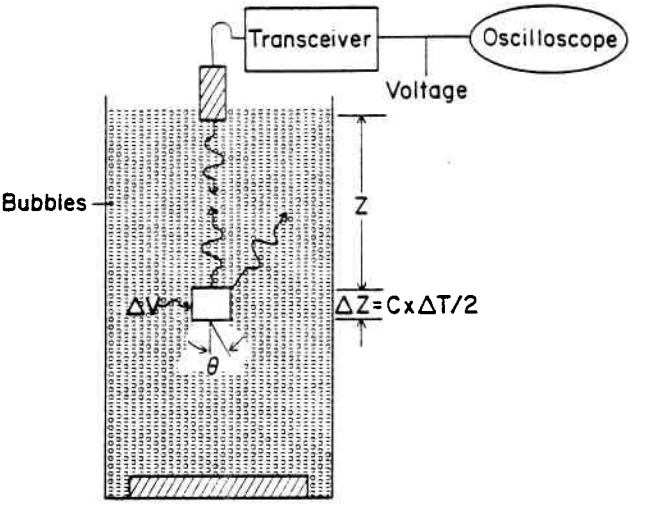


Fig. 6. Schematic depiction of apparatus for measuring reflected ultrasound signal in a beaker of contrast agent.

water, the reflected (more accurately, the scattered) wave is depicted by concentric circles propagating outward. The scattering cross-section assumed earlier for the "small mirrors" is obtained by integrating the scattered intensity over the surface of the receiver.

In Figure 5 as elsewhere, N is concentration, V is volume, K is wave number, a is bubble radius, (r, θ) is a point relative to the center of the volume in question, κ and ρ are the compressibility and density of the contrast agent ($_0$), and the gas in the bubble ($_n$). Intensity is I, either incident ($_i$) or scattered ($_s$). Cross-section is denoted by Σ. The velocity of sound in the medium (water/blood/tissue) is denoted by C; an interval of time by ΔT.

EXPERIMENTAL SETUP

A bench setup (Fig. 6) employing an ultrasound transceiver connected to an oscilloscope allowed direct recording of the radiofrequency (rf) signal. A 3.5-MHz transducer was positioned to operate in far-field in a cylinder holding a 1-liter solution of 70% sorbitol. (Sorbitol was chosen because it sustains uniformly small and stable microbubbles.) The sorbitol was sonicated serially to produce increasing microbubbles concentrations. As rf measurements were recorded, simultaneous hemocytometer counts were performed to determine the actual bubble concentration. The rf signal was recorded by taking pictures[1] of the oscilloscope at constant settings throughout the experiment. These photographs were analyzed by quantitating the largest deflection in the area corresponding to the target volume, and then normalizing so that the largest of all the deflections became 1.0. Each measured deflection is implicitly an average because the camera recorded a number of superimposed tracings.

[1]Polaroid™.

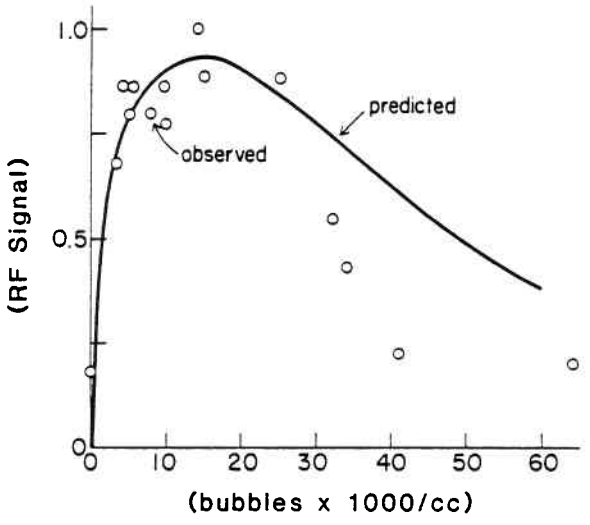


Fig. 7. Plot of radio frequency signal (normalized) versus microbubble concentration.

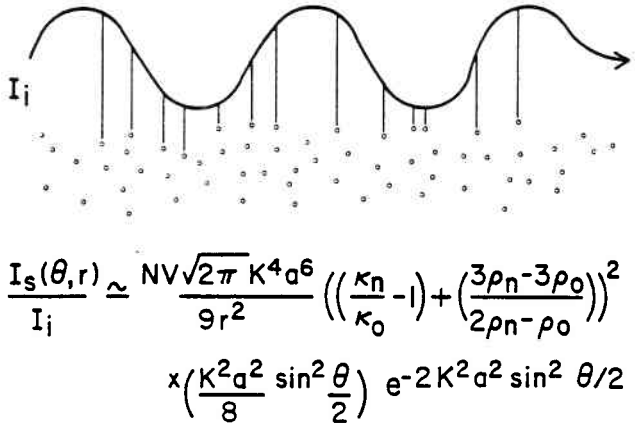


Fig. 8. Rayleigh scattering by a large number of randomly placed bubbles. Notations are the same as in Figure 5.

RESULTS

The graph in Figure 7 shows the results obtained, with normalized rf signal plotted along the vertical axis and bubble concentration plotted along the horizontal axis. The open circles represent the observed data points. The predicted curve is based on the $NK_1e^{-NK_2}$ formula derived above. The data shows a rapid rise in signal, peak region, and then a region of decreasing signal as concentration increases, as expected from the formula.

The formula also implies that the constants K_1 and K_2 can be determined from the data simply by examining the peak point. In doing so, a good fit is obtained up to a concentration limit. Above that concentration limit, the signal falls off more rapidly than predicted, owing to the assumption of single scattering. During the experiment, the sorbitol actually becomes visibly opaque owing to the large number of bubbles, violating the assumption that the ultrasound wave was scattered only once. A nonlinear least-squares fit was done to confirm the peak fit (having eliminated data affected by multiple scattering) resulting in less than a one percent change in the estimation of the peak.

FURTHER ANALYSIS

The next important factor to consider is that the bubbles are randomly arrayed along the path of the ultrasound signal (Fig. 8). Thus, instead of just multiplying the Rayleigh scattering formula by concentration times target volume, there is a diminution by factors dependent on the square of the wavenumber times the bubble radius (see [18,19] and Ishimaru [20] for treatment of more general problems of random scatters).

The random scattering intensity formulas are combined with the formula for reflected intensity by integrating the scattered intensity over a large sphere. The resultant cross-section stands in place of the assumed one when the bubbles were treated as little "mirrors." When the calculations are carried out, the experimentally observed peak would lead to a prediction of bubble radius that is within a factor of 2 of the published value, despite the calculation's dependence on not only single scattering, but the more stringent Born Approximation that the incident wave be effectively unaffected by the scattering.

DISCUSSION

If echo-contrast agents are to be used to measure regional tissue perfusion, we must have at hand a quantitative method of relating the signal received by an ultrasound scanner to contrast concentration. This paper has presented the first step towards this goal. The acoustic properties of sonicated echo-contrast agents are those of a random collection of very small scatters, the microbubbles.

The ultrasound signal reflected by the microbubbles is not simply a linear function of their concentration. There is indeed a critical concentration point above which the signal decreases. This limit can be predicted knowing the bubble size. In these calculations, a sonicated contrast agent's narrower size distribution is an advantage, in addition to the advantage that the smaller sonicated microbubbles can pass through the capillary circulation.

Other steps are required to actually achieve quantitative regional perfusion measurements. Most obvious is the construction of an ultrasound scanner capable of providing quantitative information about the signal (radiofrequency) received rather than simply a video display engineered to be pleasing to the eye. Work on this is underway, and combined with ongoing in vivo experimentation with sonicated contrast agents, this research promises to make possible quantitative regional perfusion measurements.

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REFERENCES

1. Gramiak R, Shah PM: Echocardiography of the aortic root. Invest Radiol 3:356–66, 1968.
2. Reid CL, Kawanishi DT, McKay CR, Elkayam U, Rahimtoola SH, Chandraratna PAN: Accuracy of evaluation of the presence and severity of aortic and mitral regurgitation by contrast 2-dimensional echocardiography. Am J Cardiol 52:519, 1983.
3. Kerber RE, Kioschos JM, Lauer RM: Use of an ultrasonic contrast method in the diagnosis of valvular regurgitation and intracardiac shunts. Am J Cardiol 34:722–7, 1974.
4. Feinstein SB, Ten Cate FJ, Zwehl W, Ong K, Maurer G, Tei C, Shah PM, Meerbaum S, Corday E: 2D contrast echocardiography. I. In vitro development and quantitative analysis of echo contrast agents. J Am Coll Cardiol 3(1):14–20, 1984.
5. Feinstein SB, Shah PM, Bing RJ, Meerbaum S, Corday E, Chang BL, Santillan G, Fujibayashi Y: Microbubble dynamics visualized in the intact capillary circulation. J Am Coll Cardiol 4(3):595–600, 1984.
6. Armstrong W, Mueller T, Kinney E, Tickner G, Dillon J, Feigenbaum H: Assessment of myocardial perfusion abnormalities with contrast enhanced two-dimensional echocardiography. Circulation 66:166–73, 1982.
7. Tei C, Kondo S, Meerbaum S, Ong K, Mauer G, Wood F, Skamaki T, Shimoura K, Corday E, Shah PM: Correlation of myocardial echo contrast disappearance rate ("washout") and severity of experimental coronary stenosis. J Am Coll Cardiol 3:39–46, 1984.
8. Kaul S, Pandian NG, Okada RD, Pohost GM, Weyman AE: Contrast echocardiography in acute myocardial ischemia: In vivo determination of total left ventricular "area of risk." J Am Coll Cardiol 4:1272–82, 1984.
9. Kemper AJ, O'Boyle JE, Cohen CA, Taylor A, Parisi AF: Hydrogen peroxide contrast echocardiography: Quantification in vivo of myocardial risk area during coronary occlusion and of the necrotic area remaining after myocardial reperfusion. Circulation 70:309–17, 1984.
10. Tei C, Sakamaki T, Shah PM, Meerbaum S, Shimoura K, Kondo S, Corday E: Myocardial contrast echocardiography. Circulation 66:166–74, 1982.
11. Sakamaki T, Tei C, Meerbaum S, Shimoura K, Kondo S, Fishbein M, Y-Rit J, Shah PM, Corday E: Verification of myocardial contrast two-dimensional echocardiographic assessment of perfusion defects in ischemic myocardium. J Am Coll Cardiol 3:34–8, 1984.
12. Gillam LJ, Kaul S, Fallon JT, Levine RA, Hedley-White ET, Guerrero JL, Weyman AE: Functional and pathologic effects of multiple echocardiographic contrast injections on the myocardium, brain, and kidney. J Am Coll Cardiol 6(3):687–93, 1985.
13. Santoso T, Wiratmo B, Mansyur H, Rahman AM, Panggabean M, Abduraham N: Myocardial perfusion imaging in humans by contrast echocardiography using polygelin colloid solution. J Am Coll Cardiol 6(3):612–20, 1985.
14. Goldman ME, Mindich BP: Intraoperative cardioplegia contrast echocardiography for assessing myocardial perfusion during heart surgery. J Am Coll Cardiol 4:1029–34.
15. Feinstein SB, Lang RM, Neumann A, Al-Sadir J, Chua KG, Carroll JD, Keller MW, Powsner SM, Borow KM: Intracoronary contrast echocardiography in humans: Perfusion and anatomic correlates. Circulation 72(4):III–57, 1985.
16. Frederickson ED, McCoy CE, Powsner S, Lang RM, Goldberg LI, Feinstein SB: Distribution of renal cortical blood flow measured by contrast ultrasonography. Clin Res 33:483A, 1985.
17. Rubissow GJ, MacKay RS: Ultrasonic imaging of in vivo bubbles in decompression sickness. Ultrasonics 9:225–234, 1971.
18. Rayleigh JWS: "The Theory of Sound," 2nd Edition. New York: Dover Publishers, 1945.
19. Morse PM, Ingard KV: "Theoretical Acoustics." New York: McGraw-Hill, 1968.
20. Ishimaru A: "Wave Propagation and Scattering in Random Media," Volumes 1 and 2. New York: Academic Press, 1978.