FEASIBILITY OF MITRAL FLOW ASSESSMENT BY ECHO-CONTRAST ULTRASOUND, PART I: DETERMINATION OF THE PROPERTIES OF ECHO-CONTRAST AGENTS

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(Received 6 July 1999; in final form 1 February 2000)

Abstract—Data on the ultrasonic properties of commercially available contrast agents are limited by being instrument-dependent, especially with regard to their backscattering properties. The present work describes methods of measurements that provide instrument-independent estimations of a contrast agent’s attenuation coefficient and integrated backscatter index and provide them as functions of its concentration. The two studied commercially available contrast agents were Albunex® and Levovist® SHU 508-A, both representative of agents in common use for echocardiography. The attenuation coefficients and integrated backscatter indices of both agents were found to be a linear function of their concentrations. Proportionality coefficients ± their standard deviations are provided. Actually, square root values of the averaged backscatter indices normalized with respect to the rms of the reference signal were determined. The coefficients of proportionality were found to be: $C_A = 3.11 \pm 0.1813 \text{ dB/mm}$; $C_L = 0.07 \pm 0.005 \text{ dB/mm}$ for attenuation coefficients of the Albunex® and Levovist® contrast agents, respectively, and the corresponding values for backscattering were: $D_A = 0.07 \pm 0.0054$; $D_L = 0.02 \pm 0.0012$. Being apparatus-independent, the findings of the study are important prerequisites for the use of these echo-contrast agents as an indicator in research for a quantitative assessment of blood flow. © 2000 World Federation for Ultrasound in Medicine & Biology.

Key Words: Ultrasound, Attenuation coefficient, Integrated backscatter, Echo-contrast agents.

INTRODUCTION

Applying indicator-dilution principles to echo-contrast agents assumes that it is feasible to determine the concentration of the agent by ultrasonic measurements. This, in turn, requires the knowledge of the contrast agent’s acoustic properties (i.e., attenuation and backscattering) as functions of its concentration. Several publications (Bleeker et al. 1990a, 1990b; de Jong 1993; Jayaweera et al. 1994; Skyba et al. 1994) have reported on such calibration dependencies, noting that the agents were not yet ready for use in an apparatus-independent manner. In addition to their being apparatus-dependent, video intensity-based backscattered power assessment methods are capable of providing data that can be approximated by a simple (usually linear) dependency on contrast agent concentration, but only in a very limited range of concentrations.

This part of our work describes apparatus-independent methods of measuring the acoustic properties of contrast agents as the functions of its concentration. We contend that the approach of the integrated backscatter coefficient, suggested by O’Donnell et al. (1979), which is based on solid physics, is the most appropriate one for assessing contrast agent concentrations in application to echocardiography. It is suitable for all echocardiographic equipment in which there is access to an ultrasonic signal amplitude and provides the average of backscattered power vs. concentration linear dependency for a wide range of contrast agent concentration values.

This study investigated two types of echo-contrast agents: Albunex® (Molecular Biosystems Inc., San Diego, CA) and Levovist® SHU 508-A (Schering AG, Berlin, Germany). Albunex® is available in the form of a ready-to-use suspension of air-filled bubbles in 5% water solution of albumin. The bubbles are encapsulated inside thin shells of human serum albumin. The average bubble diameter is 4.5 μm, the shell thickness is 20–30 nm and bubbles concentration is $4–5 \times 10^8 \text{ per mL}$ (de
Jong 1993; Frinking and de Jong 1998). Levovist® is supplied as granules of aggregated galactose microparticles with a very low concentration, 0.1% by weight, of palmitic acid (a physiological fatty acid). The bubbles are formed by creating a suspension of galactose microparticles in sterile water, with approximately 300 mg of galactose per 1 mL of water. When a suspension is prepared, the air contained in the voids between the microparticles is partially dissolved and produces gas saturation of the liquid volume, whereas any excess of it is released in the form of tiny bubbles with a molecular layer of palmitic acid around them. Around 95% of the microbubbles are smaller than 10 μm in diameter, and 50% of them are smaller than 3 μm.

In this study, we assume that an interaction between ultrasonic pulses and echo-contrast agent does not cause microbubble destruction. This is based on the observations of Bleeker et al. (1990a, 1990b); Cachard et al. (1996). However, recently, Wei et al. (1997, 1998) reported that such a destruction does take place, and even suggested a blood-flow assessment measuring technique based on this effect. In our experimental setup, described below (pulse repetition rate 1 kHz), we observed that averaged backscattered power and attenuation coefficient of the aforementioned echo-contrast agents do not drop below 80% of their initial values for a time period of 5 min. We infer, therefore, that microbubble destruction due to an interaction with interrogating ultrasound (US) pulses is negligible, at least in our experimental setup.

**MATERIALS AND METHODS**

The properties of the contrast agents were studied in a measuring system designed especially for this purpose (Fig. 1).

In each set of measurements, the system was filled with 500 mL of filtered (deionized) water, to which fixed doses of the contrast agent were gradually added. A magnetic stirrer was used to assure a rapid and uniform mixture of the contrast. The suspension was circulated in the system by means of a pump (Fig. 1, number 7). The distance from the probe compartment tip (Fig. 1, number 6) to the aluminum reflector (Fig. 1, number 8) was about 12 mm (volume ~22 mL).

An IPR-90 pulser-receiver IBM-PC compatible board (Physical Acoustics Corporation, Princeton, NJ) was used to stimulate the ultrasonic transducer, and to filter and amplify the signal received by the same transducer after it had been reflected from the contrast microbubbles or the aluminum reflector. The pulse duration was about 0.5 μs and the pulse repetition rate was 1 kHz. The output of the pulser-receiver was connected to the input of an IAD-90 digitizer IBM-PC compatible board (Physical Acoustics Corporation). Both boards were installed inside an IBM-386 33-MHz computer. RF ultrasonic signals were digitized with a 64-MHz sample rate and with an amplitude resolution of 8 bits (256 levels). The digitized data were stored on the computer hard disk for off-line processing.

**THEORETICAL BACKGROUND**

The intensity of an ultrasonic plane wave propagating in a direction x inside a nonhomogeneous medium, such as that of an echo-contrast agent-water suspension, decays exponentially (Morse and Ingard 1968):

\[ I(x) = I_0 \exp(-\alpha x), \]  

where \( \alpha \) is an attenuation coefficient which, in the case of a sufficiently low contrast concentration, is a linear function of the concentration (Morse and Ingard 1968):
The results of the numerical calculations of the general eqn (4) for the pressure show that, for the experimental arrangement in Fig. 1 ($F < Z < 1.16$ $F; F = 76$ mm), the radiation field of the transducer is approximately constant along the circular cylinder whose axis is identical to the transducer axis of symmetry, and that it changes abruptly in the perpendicular direction from maximum on the axis to almost zero for $r > r_1$. $J_1(kr_0r_1/Z) = 0$, and $J_1(\cdot)$ is the Bessel function of the first order. According to O’Neil (1949), the pressure field in this range of $Z$ and $r$ values is given in zero order of approximation with respect to the small value $r/Z$ by the following equation, which is exact for the case $Z = F$:

\[
p(Z, r) \equiv -ikpcv_0r_a^2 \frac{\exp(ikZ)}{2Z} \left[ \frac{2J_1(kr_0r_1/F)}{(kr_0/F)} \right] = -ikpcv_0r_a^2 \frac{\exp(ikZ)}{2Z} D(r, F). \tag{5}
\]

Using the reciprocity principle (Morse and Ingard 1968), which states that if the source of pressure $q$ at point $Q$ produces pressure $p$ at point $P$, then the source $p$ at $P$ will produce pressure $q$ at $Q$, one obtains the following relationship for the transducer response to backscattered wave from a single microbubble of radius $a$:

\[
B \propto \frac{R(a)k^2r_a^4}{Z^2} D^2(f, r_a, r, F) \exp(ik2Z - \alpha Z). \tag{6}
\]

where $R(a)$ is a reflection coefficient from a single microbubble. For an ensemble of microbubbles, this response will be:

\[
B \propto k^2r_a^4 \sum_a \frac{R_a(a)}{Z_a} D^2(f, r_a, r, F) \exp(ik2Z_a - \alpha Z_a). \tag{7}
\]
Here, the summation spreads over those microbubbles which fall inside the cylinder, $2\pi r^2 \Delta L$, parallel to the transducer axis of symmetry. This occurs because, as mentioned earlier, $D(r, F) \equiv 0$ for $r > r_1$; $J_1(kr_a r_1/F) = 0$. As to the value $\Delta L$, it stems from the fact that the ultrasonic signal is a pulse of final duration $\Delta \tau = 0.5 \mu s$, so that two waves with the phase difference $k2\Delta L > \omega \Delta \tau$ and $\Delta L > c \Delta \tau/2$ do not interfere with one another.

For low concentrations wherein the average spacing between neighboring microbubbles is higher than $\Delta L = 0.38 \text{ mm}$, eqn (6) will become:

$$B \approx \frac{k^3 r^3}{F^2} N \exp(-\alpha Z) \sum_n \frac{R_n}{N} D^2(r_n, F)$$

$$= \frac{k^3 r^3}{F^2} e^{-\alpha Z} \sum_n \frac{R_n}{N} D^2(r_n, F) \text{conc.} \quad (8)$$

Generally, however, phase differences along axis $Z$ should be accounted for by:

$$B \approx \frac{k^3 r^3 \Delta L}{F^2} e^{-\alpha Z} \text{conc.} \sum_n \sum_{nm} \frac{R_{nm}}{NM} \exp(ik2Z_m). \quad (9)$$

To make eqn (9) more mathematically tractable, it will be assumed that the distribution of the microbubbles is uniform, which means that the amplitude $B$ is a superposition of the reflections from identical cylinder slices of the contrast agent-water suspension:

$$B \approx \frac{k^3 r^3 \Delta L}{F^2} e^{-\alpha Z} \text{conc.} \sum_M \exp(ik2Z_m)$$

$$= \frac{k^3 r^3 \Delta L}{F^2} e^{-\alpha Z} \text{conc.} \int D^2(F, r)dr, \quad (10)$$

where $(R)$ is the weighted average reflection coefficient.

Under most circumstances, eqn (10) is a nonlinear relationship between the amplitude of the backscattered signal from a population of uniformly distributed contrast agent microbubbles and their concentration: the coefficient of proportionality, the sum over $M$, $\Sigma \exp(ik2Z_m)$, is dependent on $M$ and, therefore, on concentration. For a sufficiently high concentration, the summation seen in eqn (10) could be substituted by an integral over the interval $(0 - \Delta L)$, whereupon the dependency on the concentration becomes linear once more:

$$B \propto r^2 x_1 \Delta L \frac{\sin(k\Delta L)}{k\Delta L} e^{-\alpha Z}(R) \text{conc.} \int_0^{\Delta L} J_1(x) dx$$

$$\propto r^2 \Delta L \frac{\sin(k\Delta L)}{k\Delta L} e^{-\alpha Z} \text{conc.} \quad (11)$$

However, in general:

$$B \propto r^2 \Delta L e^{-\alpha Z}(R) \text{conc}(\text{conc}). \quad (12)$$

The integrated backscatter index (IBI) defined by O’Donnell et al. (1979):

$$\text{IBI} = \frac{1}{\pi} \int_0^\infty |b(t)|^2 dt$$

$$= \frac{1}{\pi} \int_0^\infty |p(t)|^2 dt$$

where $b(t)$ is the amplitude of the backscattered signal picked up by the interrogating transducer, and $p(t)$ is the amplitude of a reference signal (e.g., reflection from the aluminum plate in Fig. 1).

It can be shown (see Appendix) that the amplitude of the signal reflected from the mirror situated in the focal plane can be expressed within the precision of the first order of approximation with respect to the small parameter $r_a/F$ as:

$$A \propto r_a^2 D_1 \left( \frac{r_a}{F}, kr_a \right) \exp(ik2F),$$

where $D_1$ is a function of the parameter $r_a/F$ so that $D_1(0) = \text{constant}$, independent of the transducer dimensional parameters and the frequency.

It follows, therefore, that IBI, eqn (13), in zero order of approximation is a pure characteristic of the backscattering properties of a contrast agent, independent of the properties of the interrogating transducer, provided that the transducer aperture radius, $r_a$, is small enough with respect to its focal distance, $F$. In the first order of approximation, IBI is a weak function of the ratio $r_a/F$ and the $kr_a$ parameter:

$$\text{IBI} \propto \frac{(R_a)^2}{D_1^2} f_1(\text{conc}).$$
RESULTS

Attenuation coefficient was measured in accordance with the following equations:

\[
\frac{A_w}{A} = \exp(\alpha L); \quad \alpha = \frac{1}{L} \ln \left( \frac{A_w}{A} \right) = \frac{1}{L} \frac{20 \log_{10} \left( \frac{A_w}{A} \right)}{10^\frac{L}{20 \log_{10} e}};
\]

\[
\alpha_1 = \alpha 20 \log_{10} \left( \frac{A_w}{A} \right); \quad \alpha_1 = \frac{20 \log_{10} \left( \frac{A_w}{A} \right)}{L}, (16)
\]

where \(A_w\) is the amplitude of the mirror reflection (Fig. 1) measured in pure water, \(A\) is its counterpart measured in the presence of a contrast agent whose concentration was gradually increased, and \(L\) is the height of the water-contrast agent suspension column.

Backscattering from the contrast agent microbubbles’ signal was acquired at a maximal receiver gain setting of 60 dB. It was assumed that the signal \(b(t)\) was picked up together with the additive noise signal \(n(t)\) without being correlated with the latter, so that:

\[
B(t) = b(t) + n(t); \quad E[b(t)n(t)] = E[b(t)]E[n(t)]
\]

\[
= 0; \quad E[n(t)] = 0; \quad E[b^2(t)] = E[B^2(t)] - E[n^2(t)];
\]

where \(E\) is mathematical expectation that is substituted by the averaging of the corresponding value over the relevant time intervals.

\[
IBI, \text{ eqn (15), after filtering, therefore, will be rewritten as:}
\]

\[
IBI = \frac{\langle B^2(t) \rangle - \langle n^2(t) \rangle}{\sum_i a_i(t)}, (18)
\]

where \(a(t)\) is a momentary amplitude of the mirror reflection signal.

The overall echo-contrast agent integrated backscatter index, therefore, was evaluated as an average of those indices calculated in accordance with eqn (18) for each interval into which the whole path of ultrasonic pulse propagation in echo-contrast/water suspension was divided. Each interval corresponded to the time duration \(\Delta t = 0.5 \mu s\), and the amplitude of the ultrasonic backscattered signal on them was corrected with respect to the echo-contrast–dependent concentration attenuation (note: because the \(IBI\) values are very small, square root of the \(IBI\) was actually calculated).

Figure 3a displays a least mean square (LMS) straight line fit to the mean values of Albunex® attenuation coefficients \(\alpha_i\), \(i \leq N\), (measured with standard deviation \(\Delta \alpha_i\)), as a function of the contrast agent’s concentration.

\[
\alpha_i = C_d \times \text{conc}; \quad C_d = 3.11 \pm 0.1813 \text{ dB/mm};
\]

\[
q = 0.999; \quad p \leq 0.001; \quad N = 16. \quad (19)
\]

\(C\) was calculated in accordance with Press et al. (1988), par 14.2 as a mean value ± standard deviation (SD).

Figure 3b depicts the attenuation coefficient straight
line fit for the Levovist® contrast agent. The corresponding parameters were found to be:

\[ \alpha_L = C_L \times \text{conc}; \quad C_L = 0.07 \pm 0.0050 \text{ dB/mm}; \]
\[ q = 0.9975; \quad p = 0.0025; \quad N = 16. \] (20)

Figure 4 shows a straight line and square root fits for \( \langle (b^2(t))^{1/2} / \text{rms}(a(t)) \rangle \) values ratio calculated for Albunex®:

\[ \langle (b^2(t))^{1/2} / \text{rms}(a(t)) \rangle = D_A \times \text{conc}; \quad D_A = 0.11 \]
\[ \pm 0.084; \quad q = 0.21; \quad p = 0.79; \quad N = 16. \] (21)

In Fig. 5, analogous to Fig. 4, data are presented as they had been calculated for the Levovist® agent:

\[ \langle (b^2(t))^{1/2} / \text{rms}(a(t)) \rangle = D_L \times \text{conc}; \quad D_L = 0.07 \]
\[ \pm 0.0054; \quad q = 0.9999; \quad p \leq 10^{-4}. \] (22)

Figure 5 shows a straight line and square root fits for \( \langle (b^2(t))^{1/2} / \text{rms}(a(t)) \rangle \) values ratio calculated for the Levovist® agent:

\[ \langle (b^2(t))^{1/2} / \text{rms}(a(t)) \rangle = D_L \times \text{conc}; \quad D_L = 0.02 \]
\[ \pm 0.0015; \quad q = 0.08; \quad p \approx 0.92; \quad N = 16. \] (23)

\[ \langle (b^2(t))^{1/2} / \text{rms}(a(t)) \rangle = D_L \times \text{conc}^{1/2}; \quad D_L = 0.02 \]
\[ \pm 0.0012; \quad q = 0.999; \quad p \leq 0.0001. \] (24)

Fig. 4. Least mean square (LMS) fit of the ratio: square root of averaged integrated backscatter to the reference signal rms as a function of Albunex® concentration: (a) straight line fit; (b) square root fit.

Fig. 5. Least mean square (LMS) fit of the ratio: square root of averaged integrated backscatter to the reference signal rms as a function of Levovist® concentration: (a) straight line fit; (b) square root fit.
**DISCUSSION**

Attenuation coefficient and integrated backscatter indices were experimentally determined as functions of the concentrations of contrast agents. The methods of measurement of both parameters were shown to provide apparatus-independent estimations of the corresponding characteristics.

The attenuation coefficient was found to be directly proportional to contrast concentration for both agents, Albunex® and Levovist®, and this is in good agreement with well-known theoretically determined results for dilute solutions of scatterers-absorbers. The actually determined values of attenuation coefficients for both agents are in good agreement with the reported results of Bleeker et al. (1990a, 1990b).

Integrated backscatter indices for both studied agents were found to be very well fitted by linear functions of the concentrations of the contrast agents. This is in contrast to the rms of the backscattered signal, which was found with the same goodness of fit when given by a square root function of concentration. The actual values of IBI for both agents cannot be compared to those of Bleeker et al. (1990a, 1990b) because they were calculated (normalized) differently. However, the conclusion regarding linear dependency of IBI (not rms) on concentration is identical to that found by Wilson et al. (1993), and is supported by the conclusions of Bleeker et al., who stated that the mean video intensity (which can be assumed to be proportional to the rms of the ultrasonic signal) parameter remains directly proportional to contrast agent concentration in a very limited range, as compared to integrated backscatter index.

Efficiency of contrast agents can be evaluated using the scatter-to-attenuation ratio (STAR) described by Bouakaz et al. (1998). According to these authors, STAR is defined as:

$$\text{STAR} = \frac{\sigma_{\text{scat}}}{\sigma_{\text{ex}}} = \frac{\sigma_{c,\text{scat}}}{\sigma_{c,\text{ex}} + \sigma_{c,\text{ab}}}.$$  \hspace{1cm} (25)

Equation (25) is hard to measure. It can be substituted by the ratio of the integrated backscatter index, eqn (15), to the attenuation losses (normalized with respect to the incident intensity) that an ultrasonic signal experiences on its passage through the thickness Δl of the region from which the IBI was acquired. Δl = 0.38 mm corresponds to the pulse duration Δτ = 0.5 μs:

$$\text{STAR} = \frac{\text{IBI}}{1 - \exp(-2\alpha\Delta l)} \approx \frac{\text{IBI}}{2\alpha\Delta l}$$

$$= 10 \log_{10} \left( \frac{D_{\text{c,a}}}{C_{\text{c,a}}\Delta l} \right).$$ \hspace{1cm} (26)

where $C_{\text{c,a}}$ and $D_{\text{c,a}}$ stand for coefficients of proportionality in the eqns (19), (20), (22) and (24) or, in general, analogous to those of eqns (19), (20), (22) and (24). Because IBI as well as a were experimentally found to be directly proportional to the contrast agent concentration, this ratio provides concentration-independent and, in zero order of approximation, apparatus-independent characteristics of the corresponding contrast agent.

A calculation of STAR parameters for the Albunex® and Levovist® contrast agents yields:

$$\text{STAR}_A = 0.018$$ \hspace{1cm} (27)

$$\text{STAR}_L = 0.065.$$ \hspace{1cm} (28)

Note that eqn (28) is approximately 4 times higher than eqn (27). The reason for this is not clear. Because STAR is a concentration-independent parameter, it can be speculated that the reason lies in the difference of the distribution over the bubbles’ radii between these two agents. Compared to Levovist®, Albunex® suspension apparently contains a greater amount of relatively small air bubbles (or microparticles of a different nature) that contribute mostly to absorption rather than to scattering processes.

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APPENDIX

The pressure field of the signal being reflected from the aluminum plate, Fig. 1 can be obtained according to the concept of a specular image (Morse and Ingard 1968) as the field produced by a mirror image of the transducer placed behind the reflector, see Fig. 1A. For Z = 2F, the system of eqn (4) becomes:

\[
\begin{align*}
\frac{p}{d} &= -\frac{\nu_0 p c e^{ikF}}{d} \left\{ \exp(ik(F + d)) + \frac{r_a d}{2\pi} \sum_{n=1}^{\infty} \exp\left(ikr_a\left(r_F \sin \phi + r_F \cos \phi\right)\right) \frac{d}{r^2 - r_a^2} \sin \phi \right\} \\
&= \frac{\exp(ikr_a)}{r^2 - r_a^2} \sin \phi \\
&= \frac{\exp(ikr_a)}{r^2 - r_a^2} \sin \phi
\end{align*}
\]

Expanding the expressions dependent upon the small parameter, rF, into the corresponding Taylor series and disregarding all the items containing power of the rF parameter that are greater or equal to 2, one obtains the pressure p(Z = 2F, r) as:

\[
p = -\nu_0 p c e^{ikF} \left[ \sum_{n=1}^{\infty} \frac{r_a^2}{2\pi} \sum_{n=1}^{\infty} \exp\left(\frac{ik\left(r^2 - r_a^2\right)}{4F} \sin \phi + r_a \right) \right] \right]
\]

The amplitude of the signal reflected from the mirror, Fig. 1A, as it is sensed by the transducer, will be equal to the integral of the pressure p(Z = 2F, r) over the transducer surface S:

\[
A = \int_S p(r) dS = 2\pi \int_0^{r_a} r \left[ \int_0^{\pi} p(r) \sqrt{(dr)^2 + (dz)^2} \right] \\
= 2\pi \int_0^{r_a} r \left[ \int_0^{\pi} p(r) \right] dr
\]
\[
A = \pi r_o^2 \left( C + ik \frac{r_a}{r_o} C_1 \right) \exp(ik2F) = \pi r_o^2 D \left( k r_o \frac{r_a}{r_o} \right) \exp(ik2F)
\]

\[
C = 1 + \frac{1}{\pi} \int_0^{2\pi} d\phi \int_0^1 \frac{x \sin \phi + 1}{x^2 + 2x \sin \phi + 1} \, x \, dx; \quad x = \frac{r}{r_o};
\]

\[
C_1 = \frac{r_a}{8 \pi} + \frac{r_o}{2 \pi} \int_0^{2\pi} d\phi \int_0^1 \frac{(x \sin \phi + 1)(x^2 - 2x \sin \phi - 1)}{x^2 + 2x \sin \phi + 1} \, x \, dx; \quad x = \frac{r}{r_o}.
\]