EXPERIMENTAL AND THEORETICAL STUDY OF THE NON LINEAR PARAMETER DISPERSION OF CONTRAST AGENTS

O. Bou Matar[+], F. Vander Meulen[+], J.P. Remenieras[+], F. Tranquart [#],and F. Patat[+]
+GIP Ultrasons / LUSSI FRE CNRS 2448, Université de Tours, Tours, France
INSERM U316 / CIT, CHU Bretonneau, Tours, France.
boumatar@univ-tours.fr

Abstract
In this work, we present a theoretical and experimental study of the evolution with frequency of the nonlinear coefficient β of contrast agents. The "effective" attenuation, phase velocity and nonlinear coefficient of the contrast agent are calculated considering the encapsulating shell properties and the size distribution of gas bubbles. With a volume fraction of gas of 10^{-5}, value as high as 250 was found near the lower resonance for the β parameter, two orders of magnitude higher than biological tissues. Experimental measurements of the nonlinear coefficient β have been made in SonoVue (Bracco) and Sonazoid (Nycomed) contrast agents between 1 and 10 MHz with a second harmonic insertion - substitution method. Attenuation and phase velocity needed to the determination of β from second harmonic measurements have also been measured with the same apparatus between 1 and 20 MHz.

Introduction
Contrast agents have been found useful in many ultrasonic imaging applications such as blood flow visualisation. Most of the manufactured ultrasonic contrast agents are liquids with encapsulated gas microbubbles. Generally, the properties of the solid shell encapsulating the gas are determined by acoustic measurement of linear parameter (attenuation and phase velocity) of the studied contrast agent [1]. Nevertheless, the nonlinear coefficient is known to be more sensitive to microinhomogeneities or structural changes of a material (as phase transition) than linear parameters. However, very few measurement of the acoustic nonlinearity parameter for contrast agents have been reported [2, 3], and in each case at only one frequency (the bubbles resonance frequency). Variation of the nonlinear response of contrast agents are known to evolve with frequency, as shown for example by de Jong et al. [4]. Nevertheless, to our knowledge the nonlinear coefficient frequency evolution has not been yet measured.

In this paper, the theoretical approach used in Russian literature for nonlinear propagation in bubbly liquid is extended to the case of encapsulated bubbles mixture like contrast agents. Then, experimental measurements of linear parameters (attenuation and phase velocity) and nonlinear parameter, in the frequency range 1 to 10 MHz, are presented for two different contrast agents: Sonasoïd® and Sonovue®.

Theoretical background
Nonlinear waves in contrast agent mixture

A contrast agent mixture is a liquid with distributed encapsulated gas bubbles. It is characterised by a volume gas content *z*. In the case of low bubbles concentration (z << 1), the nonlinear wave equation in a contrast agent mixture can be expressed approximately by [5]:

$$\nabla^2 p' - \frac{1}{c_0^2}\frac{\partial^2 p'}{\partial t^2} + \rho_0 \frac{\partial^2 z'}{\partial t^2} = -\frac{\beta}{\rho_0 c_0^4}\frac{\partial^2 p'^2}{\partial t^2} \qquad (1)$$

where *p'* and *z'* are respectively the pressure and volume gas content variations, and $\beta = 1 + B/2A$ is the coefficient of nonlinearity of the fluid.

Nonlinear bubble dynamic

To obtain a closed system of equations it is necessary to relate the single-bubble volume to the pressure. To describe the behaviour of a contrast agent bubble, we assume that it maintain it spherical shape. Church [6] has obtained the nonlinear equation of motion for a gas bubble enclosed in a solid, incompressible, viscoelastic shell, given in the thin shell approximation by [1]:

$$\begin{aligned} R\ddot{R} + \frac{3}{2}\dot{R}^2 = \frac{1}{\rho_l}\Bigg[& p_{ge}\left(\frac{R_0}{R}\right)^{3\kappa} - p_\infty(t) \\ & - 4\frac{\dot{R}}{R}\left[\frac{\mu_S 3 d_{s0} R_0^2}{R^3} + \mu_L\right] - 12\frac{G_S d_{s0}}{R_0}\left(\frac{R_0}{R}\right)^4\left(\frac{R}{R_0} - 1\right)\Bigg] \end{aligned} \qquad (2)$$

where R_0 and R are the equilibrium and time dependent bubble radii, respectively. d_{s0} is the equilibrium shell thickness, p_{ge} is the equilibrium pressure in the gas inside the bubble, $p_\infty(t)$ is the pressure in the liquid far from the bubble, κ is the polytropic exponent of the gas, G_S is the shear modulus of the shell, and μ_L and μ_S are the shear viscosity of the liquid and the shell, respectively.
In term of volume displacement $V = 4/3\pi R^3$, the nonlinear bubble dynamic equation reads:

$$\begin{aligned} a\left[\frac{\ddot{V}}{V^{1/3}} - \frac{\dot{V}^2}{6V^{4/3}}\right] - \rho_l\frac{\ddot{V}}{4\pi c_0} = & \; p_{ge}\left(\frac{V_0}{V}\right)^{\kappa} - p_\infty(t) \\ & - \frac{4}{3}\frac{\dot{V}}{V}\left[\frac{\mu_S 3 d_{s0}}{R_0}\frac{V_0}{V} + \mu_L\right] - 12\frac{G_S d_{s0}}{R_0}\left(\frac{V_0}{V}\right)\left(1 - \left(\frac{V_0}{V}\right)^{1/3}\right) \end{aligned} \qquad (3)$$

where $V_0 = V(R_0)$ is the equilibrium volume, and $a = \rho_l (1/4\pi)^{2/3}(1/3)^{1/3}$.Here a radiation damping has been added by a term of the form $-\rho_l \dddot{V}/4\pi c_0$. A second order perturbation approach with $V = V_0+v$ yields:

$$\ddot{v} + 2(\beta_s+\beta_l)\dot{v} + \omega_r^2 v + \frac{R_0}{c_0}\dddot{v} = -\varepsilon p' + (\alpha+d)v^2 + \beta'(2v\ddot{v}+\dot{v}^2) + (12\beta_l + 24\beta_s)\beta' v\dot{v} \quad (4)$$

where we have introduced the following notation: $\omega_r^2 = \omega_0^2 + \omega_1^2$ with $\omega_0^2 = 3\kappa p_{ge}/\rho_l R_0^2$ and $\omega_1^2 = 12 G_S d_{s0}/\rho_l R_0^3$, $\alpha = 3\beta'(\kappa+1)\omega_0^2$, $d = 10\beta'\omega_1^2$, $\beta' = 1/8\pi R_0^3$, $\varepsilon = 4\pi R_0/\rho_l$. $\beta_s = 6\mu_S d_{s0}/\rho_l R_0^3$ and $\beta_l = 2\mu_L/\rho_l R_0^2$ are the solid and fluid viscosity damping terms, respectively. ω_r and ω_0 are the resonance cyclic frequency of the encapsulated and free bubble, respectively, and ω_1 is the shell contribution.

Second harmonic propagation in contrast agents

Consider first a transducer emitting a harmonic wave. Assuming that the energy of the second harmonic created in the medium is small, nonlinear effects do not influence the amplitude of the primary wave. In this case, the problem can be solved by the perturbation method. Combining Eqs. (1) and (4) the following Helmoltz type equations are obtained up to second order:

$$\left(\nabla^2 + \frac{\omega^2}{\tilde{c}_1^2}\right)p_1 = 0 \quad (5)$$

$$\left(\nabla^2 + \frac{4\omega^2}{\tilde{c}_2^2}\right)p_2 = \frac{2\beta_2(\omega)\omega^2}{\rho_l c_0^4} p_1^2 \quad (6)$$

with the complex propagation velocity $\tilde{c}_m$ given by:

$$\frac{1}{\tilde{c}_m^2} = \frac{1}{c_0^2} + \int_{R_{0\min}}^{R_{0\max}} \frac{\rho_l n(R_0)\varepsilon}{\left(\omega_r^2 - (m\omega)^2 + j\delta_{t_i}(m\omega)^2\right)} dR_0 \quad (7)$$

and the nonlinear coefficient of the contrast agent:

$$\beta_2(\omega) = \beta + \int_{R_{0\min}}^{R_{0\max}} \frac{n(R_0)\varepsilon^2\rho_l^2 c_0^4\left[\alpha + d - 3\beta'\omega^2 + j12\omega\beta'(\beta_l + 2\beta_s)\right]}{\left(\omega_r^2 - 4\omega^2 + 4j\delta_t\omega^2\right)\left(\omega_r^2 - \omega^2 + j\delta_{t_1}\omega^2\right)^2} dR_0 \quad (8)$$

where we have introduced the dimensionless damping constant by:

$$\delta_{t_m}(\omega) = \frac{2\beta_s}{m\omega} + \frac{2\beta_l}{m\omega} + \frac{2\beta_{rad}}{m\omega} \quad (9)$$

with:

$$\beta_{rad} = \frac{R_0(m\omega)^2}{2c_0} \quad (10)$$

So, a contrast agent mixture is an attenuating and dispersive medium, with a nonlinear coefficient depending on frequency. Introducing the liquid and the encapsulated gas bubble compressibility, $K_l = \rho_l c_0^2$ and $K_b = \omega_r^2 \rho_l R_0^2/3$ respectively, the nonlinear coefficient could be written, in the low frequency limit ($2\omega << \omega_r$) for a uniform radius mixture, as:

$$\beta_2 = \beta + \frac{K_l^2 z}{K_b^2}\left[\frac{5}{3}\frac{\omega_1^2}{\omega_r^2} + \frac{\kappa+1}{2}\frac{\omega_0^2}{\omega_r^2}\right] \quad (11)$$

Eqn (11) is equivalent to the nonlinear coefficient obtained, in the low volume concentration, by the mixture law introduced by Apfel [7] and corrected by Everbach *et al.* [8] defining the nonlinear coefficient β_{ge} of the encapsulated gas by:

$$\beta_{ge} = \frac{5}{3}\frac{\omega_1^2}{\omega_r^2} + \frac{\kappa+1}{2}\frac{\omega_0^2}{\omega_r^2} \quad (12)$$

In the case of a free gas bubble eqn (12) corresponds to the usually defined nonlinear coefficient of a gas. This mixture effect induces an increase of the nonlinear coefficient of a bubbly liquid by several orders of magnitude than the nonlinear coefficient of liquids or gas.

In the high frequency limit ($\omega >> \omega_r$) the nonlinear parameter of the contrast agent mixture tends to the nonlinear coefficient of the surrounding fluid. So, the enhanced nonlinearity introduced by the contrast agent bubbles is pronounced only in the frequency range near or lower their resonance frequency.

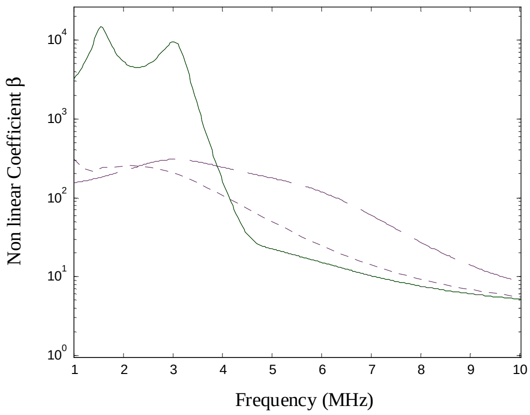

Figure 1 : Nonlinear coefficient vs. Frequency for a solution containing uniform radius R_0 = 1.2 µm free gas bubbles (solid line), uniform radius R_0 = 1.2 µm encapsulated Sonazoid® gas bubbles (dashed line), and a real radii distribution of Sonazoid® gas bubbles (dotted line) with a volume fraction $z = 10^{-5}$.

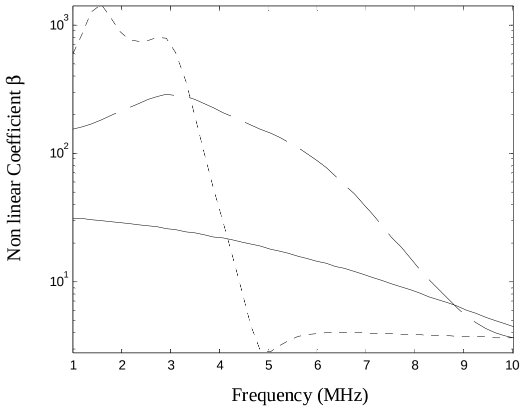

Figure 2 : Nonlinear coefficient vs. Frequency for a solution containing uniform radius encapsulated Sonazoid® gas bubbles, with R_0 = 0.5 µm (solid line), R_0 = 1.2 µm (dashed line) and R_0 = 2 µm (dotted line).

The influence of the bubble encapsulation on the nonlinear coefficient is displayed in Fig. 1: the resonance frequencies ($\omega = \omega_r$ and $\omega = \omega_r/2$) are less pronounced in mixture with encapsulated bubbles due to the enhancement of the bubble oscillation attenuation, and shifted to higher frequencies. In Fig. 2 it is shown that bigger bubbles induce higher nonlinear coefficient. This fact is confirmed in Fig 1. where the nonlinear coefficient maximum is shifted toward a smaller frequency for a real distribution Sonasoïd® mixture.

Experimental technique

Insert–substitution nonlinear coefficient measurement

A second harmonic insert-substitution method was employed to measure the nonlinearity parameter of contrast agents as a function of frequency, in the range from 1 to 10 MHz. The principle of the method, as depicted on Fig. 3, is to measure the ratio of the second harmonic pressure amplitude p_2/p_{2ref} , where p_2 and p_{2ref} are the pressure amplitudes of the second harmonic waves in the sample with unknown nonlinear coefficient β and in a reference medium with a known nonlinear coefficient β_{ref}, respectively. For nonlinear parameter measurement of contrast agent, the reference medium chosen is Isoton II the saline aqueous solution in which the contrast agent bubbles are injected.

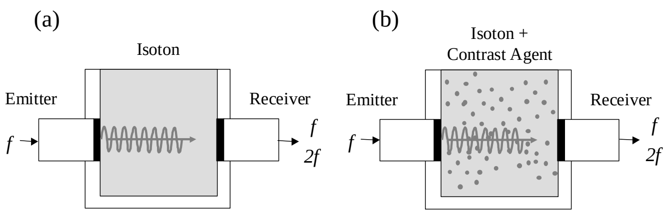

Figure 3 : Second harmonic insertion – substitution principle.

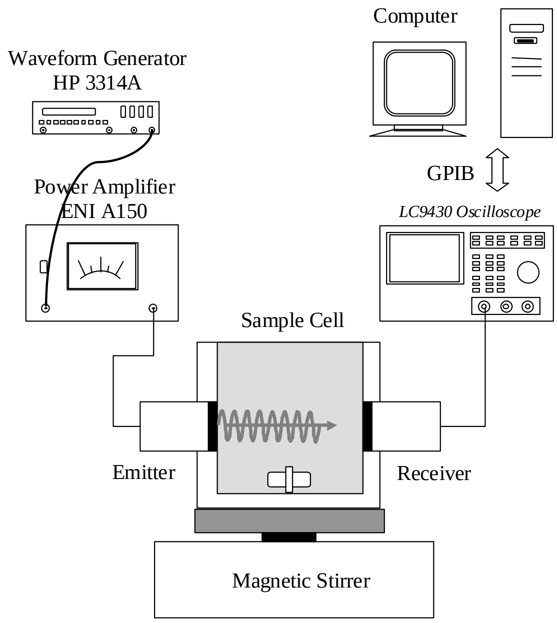

Figure 4 : Experimental set-up.

Experimental set-up

The experimental system for measuring the acoustic nonlinearity coefficient β of contrast agents at different frequencies is shown in Fig. 4. A function generator (HP 3314A, Hewlett Packard, Santa Clara, CA) produce a 50 mV amplitude sinusoidal burst signal during 20 µs, with a frequency varying between 1 and 20 MHz with a 0.5 MHz step. This signal is amplified by a 55 dB power amplifier (ENI A150, Rochester, NY) before transmission to a planar 10 MHz, 0.25” diameter PZT transducer (ISL-1002-HR, Technisonic, Fairfield, CT). The transmitted wave, which do not exceed 55 kPa, is received by a 15 MHz, 0.25” diameter wideband PZT transducer (ISL-1502-HR, Technisonic, Fairfield, CT) at a distance *d* (between 1 and 2 cm). The received signal is directly sampled by a digital oscilloscope (LeCroy 9430, Chestnut Ridge, NY), and saved on a computer via a GPIB link. In order to increase the signal to noise ratio a 128 averaging is used.

Experimental results and discussion

First, 0.06 ml of Sonazoid solution initially at $z \approx 3.10^{-3}$ has been injected in 22 ml of Isoton, giving a Sonazoid® mixture at $z \approx 9.10^{-6}$. Attenuation and phase velocity measured just after injection, and 15 min and 30 min after, are shown in Fig. 5a-b. The measures obtained just after injection are in good agreement with thus presented by Hoff [6]. Fig. 5 clearly shows a widening and a frequency shift of the resonance with time. Moreover, attenuation and dispersion become less and less important. This demonstrates an evolution of the bubbles distribution, probably linked to the disapearence of the bigger bubbles. In Fig. 5c, the nonlinear parameter measured,

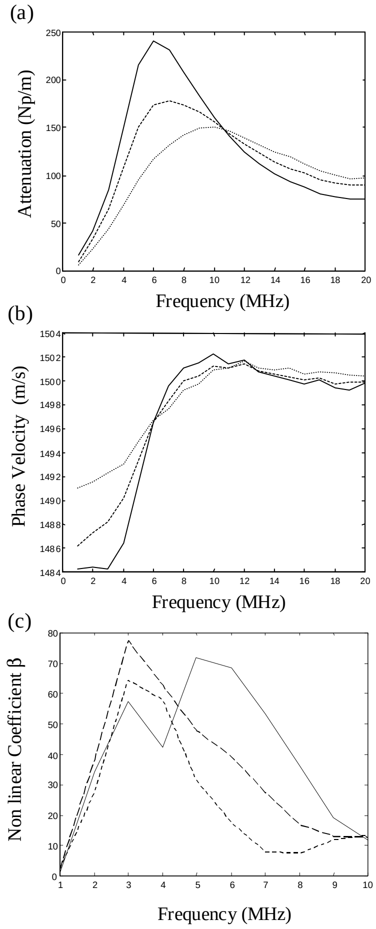

Figure 5: Measured (a) attenuation, (b) phase velocity and (c) nonlinear coefficient in Sonazoid® solution with an initial volume fraction z = 9 10^{-6}, at times t_1=0 (solid line), t_2=15 min (dashed line), and t_3=30 min (dotted line) after bubbles injection.

for the same solution at the same time, are presented. The high nonlinear coefficient dispersion predicted theoretically is confirmed. In the frequency range 1 to 10 MHz the nonlinear coefficient changes more than one order of magnitude. Moreover, the measured nonlinear coefficient peaks near or less the half resonance frequency. Nevertheless, just after injection, an unexpected maximum of the nonlinear coefficient appears near the 6 MHz resonance frequency.

Same measurements are shown in Fig. 6 for SonoVue® with an initial volume fraction of 4.5 10^{-5} and indicates similar trends.

Conclusion

The nonlinear coefficient evolution with frequency of contrast agents has been studied theoretically and experimentally. For the first time, the dispersion of the nonlinear coefficient in contrast agents has been measured. It has been shown that it peaks near or less the half resonance frequency. This parameter combined with attenuation can give more information about microbubbles size distribution.

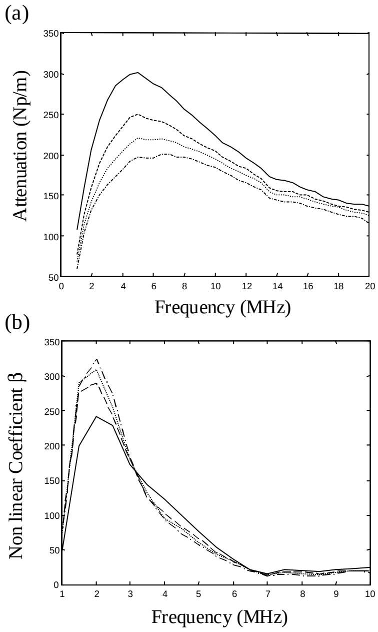

Figure 6: Measured (a) attenuation and (b) nonlinear coefficient in SonoVue® solution with an initial volume fraction z = 4.5 10^{-6}, at times t_1=0 (solid line), t_2=15 min (dashed line), t_3=30 min (dotted line), and t_3=45 min (dash-dotted line) after bubbles injection.

References

[1] L. Hoff, “Acoustic characterization of contrast agents for medical ultrasound imaging,” Ph’D Thesis, Norwegian University of Science and Technology, 2000.

[2] J. Wu and J. Tong, “Measurements of the nonlinearity parameter B/A of contrast agents,” Ultrasound in Med. & Biol., vol. 24(1), pp. 153-159, 1997.

[3] D. Zhang *et al*., “The experimental investigation of ultrasonic properties for a sonicated contrast agent and its application in biomedecine,” Ultrasound in Med. & Biol., vol. 26(2), pp. 347-351, 2000.

[4] N. de Jong, R. Cornet and C.T. Lancée “Higher harmonics of vibrating gas-filled microspheres. Part two: measurements,” Ultrasonics, vol. 32(6), pp. 455-459, 1994.

[5] E.A. Zabolotskaya and S.I. Soluyan, “Emission of harmonic and combination-frequency waves by air bubbles,” Sov. Phys. Acoust., vol. 18(3), pp. 396-398, 1973.

[6] C.C. Church, “The effects of an elastic solid surface layer on the radial pulsations of gas bubbles,” J. Acoust. Soc. Am., vol. 97(3), pp. 1510-1521, 1995.

[7] R.E. Apfel, “The effective nonlinearity parameter for immiscible liquid mixture,” J. Acoust. Soc. Am., vol. 74, pp. 1866-1868, 1983.

[8] E.C. Everbach *et al.*, “A corrected mixture law for B/A,” J. Acoust. Soc. Am., vol. 89, pp. 446-447, 1991.