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● *Technical Note*

ANALYSIS OF THE UNCERTAINTY IN MICROBUBBLE CHARACTERIZATION

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Abstract—There is increasing interest in the use of microbubble contrast agents for quantitative imaging applications such as perfusion and blood pressure measurement. The response of a microbubble to ultrasound excitation is, however, extremely sensitive to its size, the properties of its coating and the characteristics of the sound field and surrounding environment. Hence the results of microbubble characterization experiments can be significantly affected by experimental uncertainties, and this can limit their utility in predictive modelling. The aim of this study was to attempt to quantify these uncertainties and their influence upon measured microbubble characteristics. Estimates for the parameters characterizing the microbubble coating were obtained by fitting model data to numerical simulations of microbubble dynamics. The effect of uncertainty in different experimental parameters was gauged by modifying the relevant input values to the fitting process. The results indicate that even the minimum expected uncertainty in, for example, measurements of microbubble radius using conventional optical microscopy, leads to variations in the estimated coating parameters of ~20%. This should be taken into account in designing microbubble characterization experiments and in the use of data obtained from them. (E-mail: eleanor.stride@eng.ox.ac.uk)   © 2016 The Authors. Published by Elsevier Inc. on behalf of World Federation for Ultrasound in Medicine & Biology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

INTRODUCTION

Suspensions of gas microbubbles stabilized by a surfactant, protein or polymer coating have been in clinical use as ultrasound contrast agents for more than two decades (Cosgrove 2006; Cosgrove and Lassau 2010). In recent years, they have also gained renewed interest for use in tissue perfusion and local blood pressure measurements for both diagnostic and treatment monitoring applications (Andersen and Jensen 2010; Hoyt et al. 2012; Sboros and Tang 2010). Compared with X-ray angiography or magnetic resonance imaging (MRI) techniques, quantitative ultrasound imaging offers considerable advantages in terms of convenience,

cost and patient safety and, in many cases, superior specificity and sensitivity (Leen et al. 2002).

A significant limiting factor in developing more effective imaging algorithms, however, is the high degree of uncertainty in the relationship between microbubble concentration and the imaging signal (Tang et al. 2011). One source of this uncertainty is the difficulty in predicting the response of the microbubbles to ultrasound excitation. Much effort has been devoted to developing theoretical models to describe ultrasound-driven coated microbubble dynamics (Dejong et al. 1992; Church 1995; Marmottant et al. 2005; O'Brien et al. 2011; Stride 2008). Similarly, a number of highly sophisticated experimental techniques have been adapted specifically for microbubble characterization, including ultrahigh-speed video microscopy (Chin et al. 2003), flow cytometry (Tu et al. 2011) and high-frequency ultrasound measurements (Renaud et al. 2014).

Unfortunately, numerous studies have reported not only that there is considerable variability in microbubble

response across a population (Postema et al. 2005; Rademeyer et al. 2015), but also that the microbubble response is extremely sensitive to the characteristics of the sound field (frequency, pressure, pulse duration), surrounding environment (liquid density, viscosity, surface tension and presence of any boundaries) and the microbubble itself (size, gas and coating properties). Hence the results obtained from microbubble characterization experiments are likely to be very sensitive to experimental uncertainties, and this inevitably limits their utility in predictive modelling. The aim of this study was to quantify these uncertainties and their influence upon measured microbubble characteristics.

METHODS

In the majority of experimental studies, measurement is made of either the time-varying volume or radius of the bubble, $R(t)$, and/or the pressure radiated as a result of these oscillations, $p_{rad}(t)$, from which the radius can be derived (Sijl et al. 2008). These may be measured directly from individual bubbles or inferred from, for example, ultrasound attenuation or speed of sound in a microbubble suspension (Dejong et al. 1992). Given the high variability in bubble response, however, only the former are considered here.

Microbubble characteristics are determined through fitting of the experimental data to a selected theoretical model, which is typically of the form (Stride 2008):

$$RR\ddot{} + \frac{3}{2}\dot{R}^2 = \frac{1}{\rho_L}\left[\left[p_0 + \frac{2\sigma_0}{R_0} - p_v\right]\left[\frac{R_0}{R}\right]^{3\gamma}\right. \\ \left. + p_v - \frac{2\sigma}{R} - f_s - \frac{4\mu_L\dot{R}}{R} - p_0 + p_{ac}\right] \quad (1)$$

where R is the instantaneous bubble radius; the overdot denotes a time derivative, making $\dot{R}$ the velocity of the bubble wall and $\ddot{R}$ its acceleration. R_0 is the initial radius, f_s describes the influence of the microbubble coating, ρ_L is the density of the surrounding liquid, p_0 is the hydrostatic pressure, p_v is the vapor pressure inside the bubble, γ is the polytrophic constant (the gas is assumed to behave ideally), σ is the surface tension, with initial value σ_0, μ_L is the viscosity of the liquid (assumed to be incompressible and Newtonian) and p_{ac} is the pressure imposed by the ultrasound field.

Fitting may be achieved through: (i) linearization of the model to generate expressions for the amplitude and phase of microbubble oscillation, from which the unknown parameters can be determined by direct comparison with the experimental data; and (ii) optimization of the fit between the solution to the equation of motion and experimental data by varying the unknown parameters over iterative numerical solutions

(Postema et al. 2004). Given the highly non-linear nature of microbubble behavior, the latter is usually the preferred method. For the purposes of this study, we used the constitutive equation for the coating used by Hoff et al. (2000):

$$RR\ddot{} + \frac{3}{2}\dot{R}^2 = \frac{1}{\rho_L}\left[p_0\left[\frac{R_0}{R}\right]^{3\gamma} - \frac{12G_sd_sR_0^2(R-R_0)}{R^4} \right. \\ \left. - \frac{12\mu_sd_s\dot{R}R_0^2}{R^4} - \frac{4\mu_L\dot{R}}{R} - p_0 + p_{ac}\right] \quad (2)$$

The influence of the coating is described in terms of an infinitesimally thin linear viscoelastic shell characterized by its thickness, d_s, shear modulus, G_s, and shear viscosity μ_s. This selection was made in the interest of simplicity for illustration and to enable comparison with existing experimental data sets. It should, however, be noted that in the case of a surfactant-coated bubble, the concept of a shell "thickness" is somewhat misleading, as the elastic and viscous effects arise because of variations in surface molecular concentration. Hence the assignment of the value of 1 nm to d_s below does not indicate an accurate physical measure, and the quantities $G_s \times d_s$ and $\mu_s \times d_s$ could equally well be used as fitting parameters representing effective coating stiffness and viscosity, respectively (see Appendix).

To determine the effect of experimental uncertainty on the derived coating parameters, it is first necessary to estimate the magnitude of these uncertainties. There are several different sources: First, each measurement technique will have an associated uncertainty that will, in turn, affect each parameter used in the model. The initial bubble radius, R_0, is typically measured via bright-field optical microscopy and, thus, with a minimum uncertainty of ± 0.25 μm. Instantaneous bubble radius $R(t)$ measurements from high-speed video microscopy (Fig. 1) are subject to the same uncertainty. Added to this is the uncertainty due to the optical system, camera "pixel" resolution and frame rate, hence sampling frequency. In the case of laser scattering measurements, the uncertainty in $R(t)$ is approximately $\pm 0.5\%$ (Rademeyer et al. 2015). The quoted uncertainty for a calibrated hydrophone and, hence, for measurements of $p_{rad}(t)$ and the incident field $p_{ac}(t)$ varies between $\pm 5\%$ and 15% (Koukoulas et al. 2015). The hydrostatic pressure and liquid parameters are rarely reported as direct measurements in experiments, but assuming standard laboratory equipment, the uncertainty in these values can also be estimated. The parameters used for the simulations and the corresponding uncertainties are summarized in Table 1.

Sets of synthetic data were obtained by solving eqn (2) using a fourth-order Runge Kutta method