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High-speed optical observations of asymmetric pulsations of microbubbles released from tablet matrix

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Abstract: Microbubbles with a negligible shell are of utmost importance in the study of harmonic ultrasound contrast agents. The purpose of this study was to collect and quantify experimental pulsation footage of gas microbubbles released from tablet matrix under sonication. Radii were measured as a function of time of 50 microbubbles that had been released from tablet matrix and were subjected to 3-cycle pulses of 1-MHz ultrasound with a 0.3-MPa peak-negative pressure. The size distribution was measured, as well as pre- and post-sonication radii.

The experimental footage showed proof of asymmetric buckling, but also of excursion-only behaviour. The former phenomenon has been attributed to a surplus of material accumulated on the microbubble–liquid interfaces, whilst the latter phenomenon has been associated with the presence of incompressible material inside the microbubbles. The post-sonication resting radii of 41 microbubbles had become less compared to the corresponding pre-sonication radii. The opposite effect was observed with eight microbubbles in the same size range. This feasibility study confirmed that the gas microbubbles released from tablet matrix may pulsate asymmetrically. Thus, they might be suitable tracers for harmonic imaging.

Keywords: Micro-crystalline cellulose, MCC tablet matrix, controlled gas release, Rayleigh-Plesset equation, buckling.

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1 Introduction

Ultrasound contrast agents comprise microscopically small bubbles, whose radial pulsations aid in diagnostic imaging and therapeutic delivery [1, 2]. To prevent instantaneous dissolution, these microbubbles are stabilised by encapsulating shells [3, 4], as free gas microbubbles are very short-lived [5–7]. The presence of an encapsulating shell hampers the excursion amplitude of microbubbles, and thus limits harmonic imaging applications at low acoustic amplitudes [8–10]. For such purposes, microbubbles with a negligible or even without a shell would be most suited. The creation of free gas microbubbles for *in-vitro* acoustic experiments has been achieved using continuous release from micropipettes [11], ultrasound-triggered gas release from microspheres [12], and laser-nucleated acoustic cavitation [13]. The continuous release from micropipettes results in a very narrow size distribution [11], the triggered gas release from microspheres is a stochastic process [12], and laser-nucleating acoustic cavitation creates cavities undergoing rapid expansion [13]. Therefore, these three techniques are not ideal for the creation of a predictable wide size distribution of gas microbubbles.

Gas microbubble release has also been observed as a side effect of the dissolution of pharmaceutical tablets that contain entrapped gas micropockets [14]. Whether these released microbubbles can be considered free or whether their pulsation behaviour is dominated by the presence of surface-active material had thus far not been investigated. Furthermore, the symmetry of their pulsation had thus far not been studied.

Asymmetric pulsations of ultrasound contrast agent microbubbles had been observed in prior studies using high-speed photography [15–17]. So-called compression-only behaviour was reported with microbubbles that had been presumed to have excess surface material as a results of gas dissolution [15]. Expansion-only behaviour had been observed with microbubbles with incompressible core material inside the microbubbles [17].

The purpose of this study was to conduct acoustic experiments to generate pulsations of released microbubbles from tablets. The experimental data comprised preliminary high-speed video footage of tablet matrix under sonication. An ad-

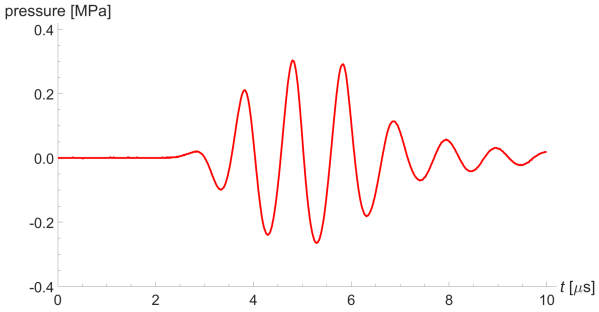

Fig. 1: Hydrophone recording of the incident pressure pulse.

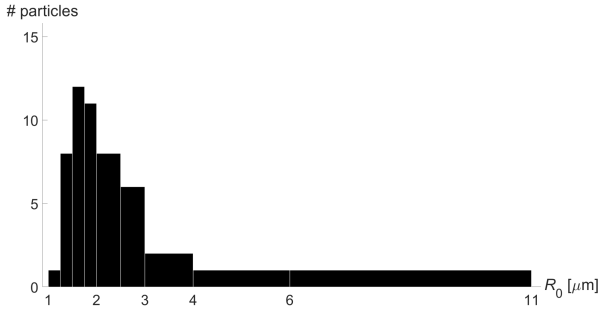

Fig. 2: Size distribution of released microbubbles pre-sonication.

ditional purpose was to identify dynamic behaviour of released microbubbles, with special attention to features less common in free microbubbles and ultrasound contrast agents.

2 Materials and methods

Avicel PH102 micro-crystalline cellulose powder (Avicel PH102, FMC Agro Ireland Limited, Cork, Ireland) underwent controlled compaction at 1.5 MPa to form tablets of 0.4-g mass, 10-mm diameter, and 4.4-mm height. This material is commonly used as tablet matrix [18]. Three fragments of 5-mg mass were manually cut off. A fragment was placed in a cylindrical compartment of 8-mm diameter and 2-mm height that was filled with degassed distilled water (FUJIFILM Wako Pure Chemical Corporation, Chuo-ku, Osaka, Japan) before being closed with an 18×18 mm Thickness No. 1 Micro Cover Glass (Matsunami Glass Ind.,Ltd., Kishiwada-shi, Osaka, Japan) and sealed with No.600M cloth tape (Sekisui Chemical Co., Ltd., Kita-ku, Osaka, Japan), so that gas microbubbles spontaneously released from the tablet matrix accumulated below the cover glass.

The compartment was part of a 244×145×76-mm^3 Perspex container that was positioned on top of an Eclipse Ti inverted microscope (Nikon Corporation, Minato-ku, Tokyo, Japan) with an S Plan Fluor ELWD 40×/0.6 objective lens focused at the bottom of the cover glass. The microscope was attached to an HPV-X2 high-speed camera (Shimadzu, Nakagyo-ku, Kyoto, Japan) [19], operating at frame rates equal to ten million frames per second during sonication with a 3-cycle pulse at a 1-MHz centre frequency and a 0.3-MPa peak-negative pressure. The pulse was generated by a custom-built focused ultrasound transducer of 65-mm diameter that was placed under a 45° angle relative to the cover glass surface and at a 60-mm distance from the optical focus, whose output, shown in Figure 1, had been calibrated in a separate setup [20].

The video data were exported to be processed offline using MATLAB® (The MathWorks, Inc., Natick, MA, USA). Segmentation was done automatically using Otsu's method for adaptive thresholding [21]. For each video, pre-sonication microbubble resting radii R_0 were determined from the first frame and post-sonication microbubble resting radii R_∞ were determined from the last frame.

The asymmetry in microbubble excursions was computed using [17]:

$$\xi = \frac{\max R(t) + \min R(t)}{R_0} - 2, \qquad (1)$$

where $R(t)$ is the measured microbubble radius as a function of time t, R_0 is the microbubble resting radius pre-sonication, and ξ is the asymmetry.

Twelve experiments were performed, generating a dataset of fifty curves of microbubble radius as a function of time.

3 Results and discussion

Figure 2 shows the size distribution of resting radii measured in the video footage. The mode resting radius was R_0=1.6 μm. More than 95% of the microbubbles had resting radii $R_0 \leq 4.0\,\mu$m. This distribution is similar of form to those published on lipid-shelled ultrasound contrast agents [3].

Figure 3 shows an example of an experimental $R(t)$ dataset of a microbubble of a 1.4±0.1-μm pre-sonication radius. The microbubble was not observed to expand at all during sonication, whilst the contraction amplitude was greater than 0.4 μm. The pulsation behaviour appeared very similar to that described in literature [15, 16]. This result indicates that there was an excess of surface-active material present on the interface of the microbubble.

Figure 4 shows another example of an experimental $R(t)$ dataset of a microbubble of a 1.5±0.1-μm pre-sonication radius, *i.e.*, the same initial radius as above. Here, contraction was hardly observed, whilst the outward excursion approximated 1 μm. There is similarity with asymmetric outwards

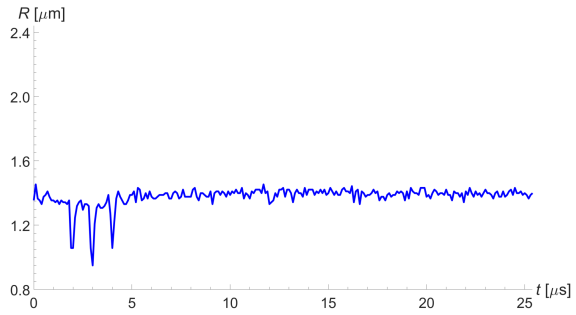

Fig. 3: Radius as a function of time of a microbubble of 1.4±0.1-μm pre-sonication resting radius, showing compression-only pulsation behaviour.

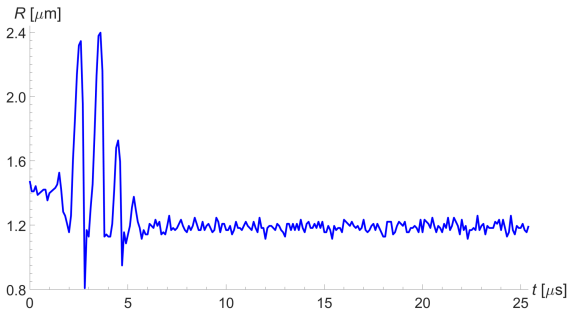

Fig. 4: Radius as a function of time of a microbubble of 1.5±0.1-μm pre-sonication resting radius, showing expansion-only pulsation behaviour.

excursions described literature [17]. This result indicates that there was incompressible material present inside the microbubble. The microbubble was measured to have a 1.2-μm resting radius post-sonication. This might be the result of surface material that had been either released from or redistributed on the microbubble surface.

Figure 5 shows an experimental $R(t)$ dataset of a microbubble of a 5.7-μm resting radius pre-sonication. This microbubble was observed to expand and contract. The contraction amplitude was greater than the expansion amplitude, however. The high-speed footage showed buckling during bubble contraction, whilst outward expansion appeared to be spherically symmetric.

This experimental footage showed proof of asymmetric buckling. This phenomenon has been attributed to a surplus of material accumulated on the microbubble–liquid interfaces.

Figure 6 shows the asymmetry in oscillation amplitude as a function of the pre-sonication resting radius. The results differ greatly independent of resting radius, with most microbubbles showing either positive or negative asymmetry, and only two microbubbles showing symmetric excursion amplitudes. These results indicate that surface-active materials from the tablet matrix might have accumulated on the microbubble interfaces, but also inside the microbubbles, creating incompressible cores.

Figure 7 shows the asymmetry in oscillation amplitude as a function of the pre-sonication resting radius. The post-sonication resting radii of 41 microbubbles had shrunk compared to the corresponding pre-sonication radii. The opposite effect was observed with eight microbubbles. Any conclusion on the integrity of the microbubble surfaces is premature.

4 Conclusions

This feasibility study confirmed that the gas microbubbles released from tablet matrix pulsate asymmetrically. Thus, they might be suitable tracers for harmonic imaging.

Author Statement

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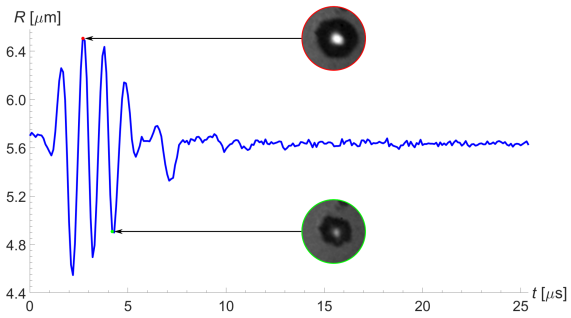

Fig. 5: Radius as a function of time of a microbubble of 5.7-μm pre-sonication resting radius, and selected high-speed footage overlain. The overlain frames correspond to 20-μm diameters. The microbubble captured during contraction (green) showed buckling.

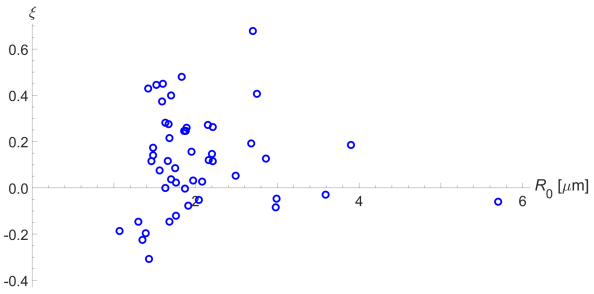

Fig. 6: Asymmetry in microbubble pulsation excursion as a function of pre-sonication resting radius.

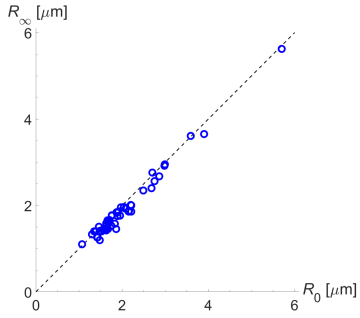

Fig. 7: Post-sonication resting radius as a function of pre-sonication resting radius.

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