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# Oil-filled polymeric ultrasound contrast agent as local drug delivery system for lipophilic drugs

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*Abstract*—**Novel polymeric microcapsules, filled with a mixture of gas and oil, were produced and their potential as ultrasound contrast agent-based drug delivery system for lipophilic drugs was investigated. Microcapsules were synthesized that contained either no oil, were almost half-filled with oil, or were almost completely filled with oil. Mean number weighted diameters were between 1.22 and 1.31 μm. At a low MI (1 MHz, P_ of 0.24 MPa), microcapsules typically compressed without cracking. At a high MI (1 MHz, P_ of 0.51 MPa), microcapsules cracked, thereby releasing their content. Guidance and monitoring of therapy will be possible because the microcapsules were echogenic and stable at low MI. These microcapsules therefore have great potential as local drug delivery system for lipophilic drugs.**

***Keywords – polymeric ultrasound contrast agent; local drug delivery; lipophilic drug; diagnostic ultrasound***

## I. INTRODUCTION

Ultrasound contrast agents (UCA) are routinely used in perfusion imaging in cardiology and radiology [1]. Over the recent years, lipophilic as well as hydrophilic (model) drugs have been incorporated in UCA. The advantage of using UCA as drug delivery systems is the local and triggered release of a therapeutic at the region of interest only. In addition, ultrasound imaging will aid the guidance of therapy. However, loading sufficient amount of therapeutic agent onto or into the coating of UCA as well as its efficient release are current limitations for these UCA-based drug delivery systems [1-3].

This study focuses on the production and characterization of novel near-micrometer polymer-shelled microcapsules designed for the local delivery of lipophilic drugs. In principal there are several ways to incorporate drugs into UCA. In this study we dissolved the lipophilic drug in oil, i.e. hexadecane, and loaded this within the microcapsules. The aim was to use diagnostic ultrasound to release the oil so that the incorporated drug is released from a solution or a fine dispersion rather than through diffusion from the shell of the polymer microbubbles, as the latter has been shown to be inherently slow [4]. Preliminary *in vitro* and *in vivo* studies already suggested the potential of these novel polymeric half oil-filled microcapsules as a UCA-based drug delivery system [5]. Important characteristics, such as the acoustic response, the mechanism of US-triggered drug release, and the effect of the amount of encapsulated oil on the acoustic properties were not investigated yet. In this study, we investigated the efficiency to incorporate oil in the microcapsules, the particle size distribution, morphology, acoustic attenuation properties, US-triggered drug release, and US imaging capacity.

## II. MATERIAL AND METHODS

### A. Polymeric UCA preparation

The polymer poly(L-lactic acid) terminated with 1H-1H perfluoro-octan-1-ol (MW 3,000), abbreviated as pLA-pFO, formed the shell of the microcapsules [6]. For a full description of the preparation, see [7]. In brief, a solution of the shell-forming polymer and an alkane in dichloromethane (DCM) was prepared and emulsified in water containing poly-vinylalcohol. This solution was shaken manually to prepare a premix and then repeatedly pressed through a glass filter. Microcapsules were prepared with cyclodecane and hexadecane at a ratio of 1:0 for the production of completely gas-filled microcapsules (Sc), at a ratio of 1:1 for the production of half oil-filled microcapsules (Sch), and at a ratio of 0:1 for the production of almost completely oil-filled microcapsules (Sh). As a reference, solid polymer particles were prepared from the pLA-pFO solution in DCM without the addition of alkanes. Microcapsules were freeze-dried to remove the cyclodecane from the core. The absorbing dye Sudan Black is a hydrophobic molecule and was chosen to mimic a lipophilic drug. Sudan Black was incorporated into the microcapsules by adding 0.52 mg (Sc and Sh) or 0.26 mg (Sch) Sudan Black to the solution of pLA-pFO in DCM during the microcapsule preparation.

### B. Composition of polymeric UCA

Efficiency of hexadecane encapsulation was determined using Gas Chromatography/Mass Spectrometry (GC/MS). The amount of encapsulated gas was determined with oscillating U-tube densitometry with a DMA 5000 (Anton Paar). Microcapsule size distributions were measured on a Coulter

Counter Multisizer 3. Morphology was determined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

### C. Acoustic attenuation properties of polymeric UCA

Attenuation as a function of frequency was measured *in vitro* to reveal acoustic properties of the microcapsules. A pulse-echo set-up was employed using the back wall of the sample chamber as a reflector. In a water tank, four focused single element transducers were mounted in parallel to allow attenuation measurements from 0.3-22 MHz on the same sample. The input of the transducers was 1 cycle of a sine at the centre frequency, which resulted in short pulses with an MI of 0.033. For each transducer, the attenuation was calculated at -20 dB bandwidth around the centre frequency and divided by the acoustic path length. Microcapsule and solid polymer particle concentration was $3.5\times10^5$/ml in the 430 ml sample chamber.

### D. Characterization of UCA behavior and drug release

Microcapsule behavior upon insonification (1 MHz, 10-cycle-sine wave bursts, P_ ranging from 0.17 to 0.51 MPa) was studied in detail with the Brandaris 128 high-speed camera [8]. Images of insonified microcapsules were recorded in six sequences of 128 image frames at a speed ~ 10 million frames per second. An area-time curve was extracted from the recorded images. Ultrasound-triggered drug release (1 MHz, 1-cycle-sine wave burst, P_ 0.51 MPa) was optically studied using a high sensitivity CCD camera (LCL-902K, Watec) at video frame rate (30 frames/sec).

### E. Ultrasound imaging

Using a commercial echocardiography ultrasound system (GE/VingMed System 5), fundamental B-mode images were recorded of both a tissue-mimicking phantom and microcapsules at 2.5 MHz and P_ varying from 30 to 530 kPa.

## III. Results

### A. Composition of polymeric UCA

Microcapsules were synthesized that contained either no oil (Sc), were almost half-filled with oil (Sch), or were almost completely filled with oil (Sh), see Table 1. Loading them with different amounts of oil did not influence their size distribution. More than 99% of the microcapsules were less than 3.04 μm in diameter. TEM revealed that Sc microcapsules were hollow, and had a thin shell of ~40 nm. SEM revealed that Sc microcapsules were spherical and had an uneven surface. Morphology of Sch and Sh microcapsules could not be studied since these broke upon introducing them into the vacuum column of the electron microscopes.

### B. Acoustic attenuation properties of polymeric UCA

Attenuation was highest for Sc microcapsules. Maximum attenuation for Sc was around 20 MHz, while that for Sch and Sh was around 13 MHz, indicating that oil encapsulation lowered the resonance frequency. The attenuation of the solid polymer particles was ~0 dB/cm over the entire frequency range, indicating that they do not contain gas.

TABLE I.

| Sample label | Composition of microcapsules | | |
|---|---|---|---|
| | *Calculated fill of microcapsules with hexadecane (= oil)*[a] | *Encapsulated gas per microcapsule (% v/v)*[b] | *Number weighted mean diameter (μm)*[c] |
| Sc | 0% | 88.8 ± 9.2 | 1.21 |
| Sch | 40-42% | 34.9 ± 0.7 | 1.22 |
| Sh | 97-100% | 9.7 ± 0.6 | 1.31 |

a. Determined with GC/MS; b. determined with densitometry; c. determined with Coulter Counter

### C. Characterization of UCA behavior and drug release

Brandaris 128 high-speed camera recordings enabled us to optically observe how individual microcapsules behaved when insonified at a frequency of 1 MHz. At a low MI (0.17 MPa < P_ < 0.24 MPa), the three differently loaded microcapsules typically compressed without shell cracking. An area-time curve constructed from a recording of an Sc microcapsule, 1.2 μm in diameter, is given in Fig. 1 (top panel). Compression of the microcapsule was clearly visible in frame #32 and #70 as illustrated in the bottom panel of Fig. 1. The compression was not symmetrical, and correlated with a positive pressure of the acoustic cycle. These compressions did not crack the microcapsule as it was still intact in the first frames of the next recording which was recorded 80 ms later. However, in this

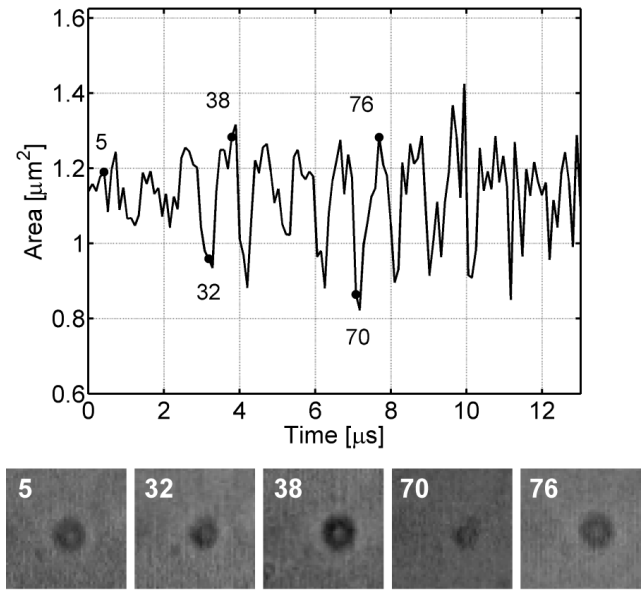


Figure 1. Brandaris 128 high-speed camera recording of an Sc microcapsule, 1.2 μm in diameter, insonified with a 1 MHz transducer at P_ of 0.17 MPa. Top panel: area-time curve. Bottom panel: five selected cropped frames (4.5 by 4.5 μm) out of total of 128 frames showing the microcapsule before insonification (frame #5) and during insonification (#32, 38, 70, and 76). Compression but no cracking is observed.

next recording, in which the P_ was increased to 0.24 MPa (MI = 0.24), this microcapsule did crack after a few more compression cycles since we observed gas escaping. Similar observations were made for Sch and Sh microcapsules (see [7]). Although compression behavior was identical for Sc, Sch, and Sh microcapsules, the onset was at different acoustic pressures. More Sc microcapsules compressed at the lowest P_ of 0.17 MPa compared to Sch and Sh microcapsules. Also, more Sc microcapsules cracked at a lower P_ than did Sch and Sh microcapsules. At a high MI (P_ of 0.51 MPa; MI = 0.51), Sc, Sch, and Sh microcapsules typically compressed once, leading to cracking or at least weakening of the shell of the microcapsule since in the following negative pressure of the acoustic cycle we observed gas escaping from the microcapsules. This is illustrated in Fig. 2 in which nine selected frames out of 128 frames are shown from a typical Brandaris 128-high speed camera recording of an Sc microcapsule with a diameter of 2.0 μm (see frame #4). The microcapsule compressed (frame #10-12) at a positive pressure of the acoustic cycle and returned to its original spherical shape (frame #15) when the pressure was zero. In the following negative pressure of the acoustic cycle, gas escaped from the microcapsule (frame #16-20). At the peak positive pressure, the free gas bubble was so compressed that it was no longer visible (frame #23). During the following ultrasound cycles, the free gas bubble continued to expand and collapse. The free gas bubble was no longer visible in the successive recording which was taken 80 ms later, indicating that it had dissolved. At high MI, the behavior of Sch and Sh microcapsules (see [7]) was similar as described for Sc microcapsules.

For drug release studies, microcapsules loaded with Sudan Black were used. Drug release was triggered using one burst of a 10 cycle-sine wave at a P_ of 0.51 MPa (MI = 0.51). Normal video recordings of Sch and Sh microcapsules showed that the shell of the drug-loaded microcapsules cracked upon insonification, thereby releasing the encapsulated drug. Shell fragments as well as the released oil droplet were identified. When Sc microcapsules were sonocracked, only the fragmented shell was observed (see [7]).

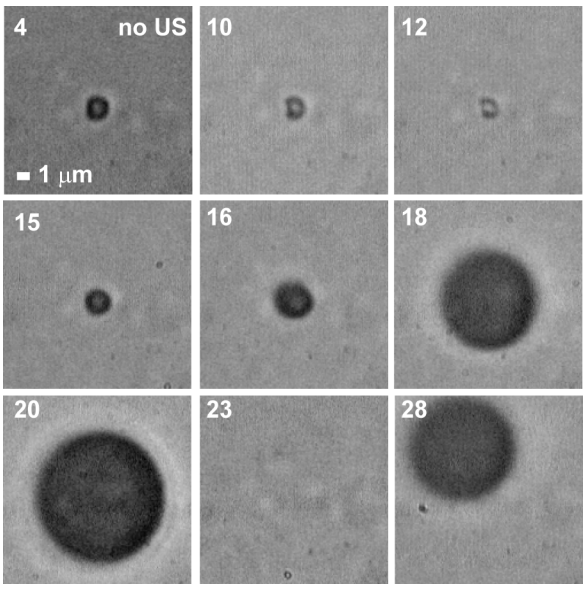


Figure 2. Nine selected cropped frames of a Brandaris 128 high-speed camera recording of an Sc microcapsule, 2.0 μm in diameter, insonified with a 1 MHz transducer at P_ of 0.51 MPa.

### *D. Ultrasound imaging*

Fundamental B-mode images recorded with the GE/VingMed System 5 revealed that all three differently loaded microcapsules showed marked scattering over a tissue-mimicking phantom (data not shown), indicating that guidance and monitoring of therapy will be possible.

## IV. Discussion

For UCA-based drug delivery systems, it is important that the microbubbles incorporate an efficient payload of the drug [2, 9-12]. The polymeric microcapsules produced in this study contained distinctly different amounts of oil and gas. Summing the amount of oil and gas for the Sc, Sch, and Sh microcapsules resulted in values rather close to 100% (79-111%). By incorporating the oil, which serves as a hydrophobic drug-carrier reservoir, the pharmaceutical payload per bubble will be dramatically higher than when drugs are attached to or incorporated into the shell of microbubbles [2]. This approach was also reported for lipid-coated microbubbles (acoustically active lipospheres, AALs), in which soybean oil [13] and triacetin oil [14] were used. Although the Sch and Sh microcapsules described in this study have even larger drug carrier reservoirs than the AALs, the therapeutic significance of this needs to be investigated. Furthermore, the payload will depend on the amount of drug that can be dissolved in oil.

For UCA-based drug delivery systems, it is also important that the microbubbles can be triggered to release the drug with diagnostic ultrasound [2, 9-12]. The oil-loaded microcapsules used in this study typically cracked, thereby triggering drug release, at an MI of 0.34 and 0.51, which is within the clinically safe dose of diagnostic ultrasound and can be generated by regular ultrasound diagnostic equipment. In comparison, ultrasound-triggered drug release from AALs loaded with triacetin is at higher MI's, as values of 0.67 to 2.0 have been reported [14-16]. Moreover, AALs loaded with soybean oil were even more difficult to fragment [14]. It is also striking that much lower MI's are required to crack our polymer-shelled microcapsules in comparison to most other polymer-shelled microbubbles [17-20].

In addition, it is important that UCA-based drug delivery systems can be imaged under non-destructive conditions so that ultrasound can be used for guidance and monitoring of therapy [2,9-12]. All three different loaded polymer-shelled microcapsules described in this study are suitable for low MI imaging, which is an important finding. At low acoustic pressures, compression-only behavior was observed, which was comparable to behavior found for the polymer-shelled microsphere PB127 [20].

Although the Sch and Sh microcapsules contained less gas than the Sc microcapsules, the resonance frequency of the Sch

and Sh microcapsules was lower than for the Sc microcapsules. This was unexpected since it is known that smaller gas bubbles have higher resonance frequencies than larger gas bubbles [21]. The discrepancy may be explained by the presence of oil and a relatively higher shell to gas ratio which may have increased damping which would result in a lower resonance frequency [22]. In addition, hexadecane may have influenced the pLA-pFO shell as we observed in the MDSC thermogram [7] that the shell was less crystalline when only hexadecane was loaded in the microcapsules. This may have made the shell less rigid which may also have contributed to the lower resonance frequency. Further studies are needed to elucidate this.

For Sc, Sch, and Sh microcapsules we previously reported [5] single microcapsule activation as a function of P_ using event counts. When insonified at 1 MHz (signal detected from 3-7 MHz), all three differently loaded microcapsules showed a threshold followed by a sharp increase in event count for increasing P_. Sh microcapsules had the highest threshold. These observations are in agreement with the results of the Brandaris high-speed camera recordings at low MI reported here.

Although we have demonstrated US-triggered release with our partially oil-filled microcapsules loaded with Sudan Black, incorporating actual drugs and quantifying their release need to be investigated and will be the focus of our future research. At the same time, preliminary *in vivo* studies with Sch microcapsules loaded with the chemotherapeutic drug paclitaxel were encouraging [5], but further studies need to be done to demonstrate the therapeutic effect of this novel UCA-based drug delivery system *in vitro* as well as *in vivo*.

In conclusion, the novel oil-filled polymeric ultrasound contrast agents produced and characterized in this study have great potential as a local drug delivery system for lipophilic drugs.

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