Ultrabubbles: Ultrasound-Responsive Microcapsules

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ABSTRACT

An approach is presented to fabricate organosilica/silica capsules with control of size, shell thickness and shell elasticity. The capsules are fabricated by a sacrificial template mechanism, using a modified polystyrene latex particle as the template, and an organosilane matrix for the shell. Following core dissolution, the cavities are filled with air rendering the capsules sensitive to insonation. Their application as ultrasound contrast agents is demonstrated together with preliminary data that provides evidence of controlled payload release under acoustic pressure.

Keywords: microcapsule fabrication, hollow particles, ultrasound contrast agent, controlled-release systems.

1 INTRODUCTION

Ultrasound has been used as an imaging modality and for its therapeutic capabilities for decades. High intensity focussed ultrasound heats and destroys pathogenic tissue in the treatment of cancer[1,2,3] and hemostasis[4,5]. High energy ultrasound is used in medical environments for the treatment by ultrasound-induced hyperthermia[6], and transdermal drug delivery is often used in conjunction with ultrasound to provide a greater efficiency of drug uptake[1]. In some instances the use of ultrasound contrast agents (UCA) greatly improves the technique for both imaging and drug delivery.

Early bubble UCA's to be approved by the U.S Food and Drug Administration (FDA) comprised of human serum albumin (Albunex® and Optison™) or lipid stabilised (Sonovue™) gas bubbles. More recently, protein and lipid stabilied microbubbles have been functionalised for their use as delivery vehicles, to carry targetting ligands[7] and payloads[8,9].

We have reported the development of a novel laminated organosilica/silica based contrast agent[10], which overcomes the storage and shelf-life stability issues associated lipid and human serum albumin based UCA's. These new UCA microbubbles, stabilised by a tailored multilayered silica and organosilane matrix, can be tuned to show resistance to cavitation, whilst providing a large acoustic backscatter within a wide acoustic pressure range (M.I 0.4 – 1.9). In this manuscript we link the capsule fabrication process and their acoustic properties, which enable their use as UCA's. Preliminary data is introduced to demonstrate how the lamination can be tailored to influence the acoustic pressure threshold for inertial cavitation. This system potentially provides a means to visualise the UCA's whilst also enabling the release of an encapsulant at higher acoustic pressures, showing potential as a drug delivery candidate.

2 EXPERIMENTAL

2.1 Materials

4-styrenesulfonic acid, poly(acrylic acid) (PAA; Average MW ~ 450000, poly(allylamine hydrochloride (PAH; Average MW~56,000), styrene ≥99%, octyltriethoxysilane (OTES; ≥98%), bis[3-(trimethoxy-silyl)-propyl) amine (TSPA; 90+%) 2-propanol, absolute ethanol, ammonia and tetrahydofuran (THF) were all obtained from Sigma-Aldrich. (*3-mercapto-propyl*) *trimethoxysilane* (MPTMS; ≥97%), Tetraethoxysilane (TEOS; ≥98%) and 2,2'-*azobisisobutyronitrile* (AIBN) were all purchased from Fluka. All solutions were kept at 5°C in a refrigerator. Standard salt solutions, HCl and NaOH solutions were prepared in distilled water

2.2 Instrumentation

The scanning electron microscopy (SEM) images were taken using a FEI Phillips XL30 SEM operating at 5KeV. For conventional imaging, the samples were sputter coated with a 4nm layer of gold (Quorum EmiTech, K575X), and for energy-dispersive x-ray spectroscopy the samples were coated with a thin film of carbon. The transmission electron microscopy (TEM) images were taken using a FEI Phillips CM100 TEM. A 1mg/L suspension of the particles and capsules were deposited and dried on 400 mesh, holey carbon, film grid (Agar Scientific). Particle and capsule size analysis was obtained using a Malvern Zetasizer 3000. A solution of 5 x 10^8capsules/L were used throughout the ultrasound experiments. Validation of the capsules as contrast agents was carried out using a Siemens Asucon Sequola 512 with a 2MHz transducer. Investigation of their application as delivery vehicles, a Sonix RP ultrasound system fitted with a 5MHz transducer was used. Mechanical Index (M.I, defined as the peak rarefactional pressure divided by the square root of the ultrasound frequency) was used as a measure to study the capsules response to acoustic pressure.

3 RESULTS AND DISCUSSION

3.1 Ultrabubble fabrication

The fabrication of the 'Ultrabubble' microcapsules follows a sacrificial template sol-gel mechanism presented by Lin et al[10] This requires (i) a modified template particle, (ii) silica and organosilane shell growth, (iii) the selective removal of the template particle. In the final step (iv) the capsule core can to be filled with gas and re-suspended into water

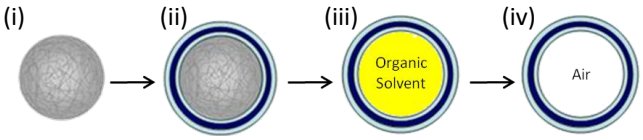

Figure 1. A schematic of 'Ultrabubble' fabrication

(i) Fabrication of Modified Template Particle

Since diameter of the template particle determines the final diameter of the capsule core, polystyrene particles were chosen, owing to their ease of fabrication, control of particle size and solubility in organic solvents, enabling removal of the template core after shell formation. To produce particles with a diameter of approximately 2.4μm, styrene (4.6g), 4-styrenesulfonic acid (0.037g), PAA (0.226g) and AIBN (0.1g) were dissolved in absolute ethanol (13.4mL) and UHP water (0.69mL). The mixture was stirred at RT under nitrogen for 30min at 120rpm and then heated to 80°C for a further 23.5h. The product was washed in ethanol under repeated cycles of centrifugation-re-suspension at 3,000rpm and dried. The size of the template particle can be adjusted by changing the concentration and ratio of styrene and PAA added during polymerisation. Larger particles require more styrene and PAA is adjusted to maintain the right viscosity to avoid particle precipitation during polymerisation. The prepared particles have an inherent negative charge with low charge density. This produces poor silica coating and incomplete shells. To produce a positive surface charge suitable for sol-gel nucleation, the particles were modified with PAH (1% PAH in water), sonicated for 30 minutes and washed in UHP water under repeated cycles of centrifugation-re-suspension at 3,000rpm and dried under vacuum for 24 hours.

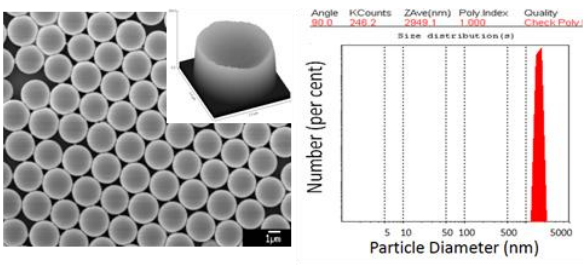

Figure 2. (a) A scanning electron micrograph of the PAH-modified PS particle (inset) a generated topograph map of an individual particle (b) Zetasing data of the particles hydrodynamic diameter.

Scanning Electron Microscopy revealed a mean particle diameter of 2.38± 0.08μm, whereas zeta sizing suggested a mean diameter of 2.95μm (figure 2) The discrepancy is characteristic of the analysis method, with the particles measured under vacuum in SEM giving a smaller volume than the hydrodynamic diameter measured in solution by zeta sizing.

(ii) Silica and Organosilica Shell Growth

The formation of an organosilica shell on a solid particle template was adapted from Lu et al[11] with further modifications of the Stôber method[12]. The hydrolysis and polycondensation of organosilanes into organosilica matrices is a proven technique and in principle can be used to produce a shell with sufficient elasticity to raise the inertial cavitation threshold. However, with TSPA and OTES for example, limitations arise since the same material properties that result in the elasticity tend to result in aggregation. Introduction of a thin overlayer of TEOS prevents this aggregation, without inhibiting the elasticity of the inner organosilane sol-gel. The fabrication procedure requires dried polystyrene particles (0.256 g) to be mixed with silane monomers, TEOS, OTES and TSPA in 2-propanol (197 mL), UHP water (34.4 mL), ammonia (5 mL). The concentration of silane used could be varied depending on required shell thickness and overall shell thickness could be controlled by both silane concentration and the time for the reaction. Copolymerization of TSPA (0.62mM), OTES (9.68mM) and TEOS (3.26mM) were demonstrated to form capsules successfully. pH during polymerization was also important. 1 M HCl was used to adjust the overall pH to below 11. The reactant was stirred at room temperature for 1.5–5 h depending on the silane. The product was washed in 2-propanol for repeated cycles with centrifugation (3500 rpm).

To tune capsules to rupture at higher acoustic pressures, a greater concentration of TEOS is required in relation to the organosilanes. TEOS is known to yield brittle, less elastic silica shells, which are likely to be more susceptible to rupture during rarefaction of the cavity upon insonation. In this instance the fabrication procedure for the shell requires for example, PS (0.2g) to be mixed TEOS (1ml) with ammonia (4ml) in 2-proponol (176mL) and UHP water (26.9mL) for 1 hour at 300rpm. MPTMS (0.454ml) was added to the mixture, followed by TEOS (0.776ml) at 1.5 hours; the reaction was then left for a further 1.5 hours and the product washed in 2-proponol under repeated cycles of centrifugation-re-suspension at 4,500rpm. The resultant particles comprise of a polystyrene core and a 3-layer silica/organosilica/silica shell (Fig 3).

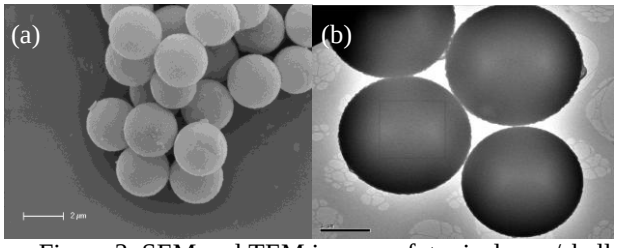

Figure 3. SEM and TEM images of typical core/shell particles developed through this process.

The core/shell particles are monodisperse with TEM and SEM imaging reveal a mean diameter of of 2.58±0.08um, estimating a shell thickness of 100nm. TEM also shows the particles to have an opaque core indicating the presence of the solid core

(iii) Removal of Template Particle

The organosilica/silica shells prepared using this approach have been reported to be highly porous[13]. This allows the PS core particle to be dissolved through relatively thick silica[13] and organosilica[10] shells by successive washes in organic solvents such as toluene and THF. The core/shell particles were incubated in THF for 24 hours and then centrifuged at 4,000rpm. The precipitate of THF-filled organosilica capsules was re-suspended in THF and washed for 6 cycles of 2 hours.

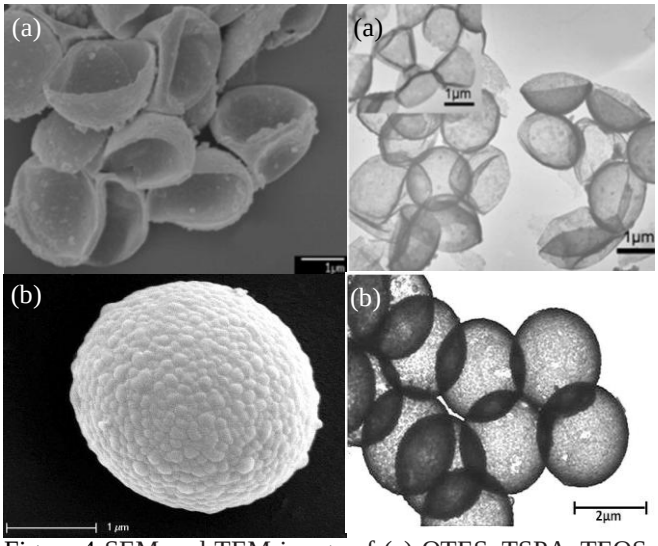

Figure 4 SEM and TEM image of (a) OTES–TSPA–TEOS composite hollow particles and (b) TEOS-MPTMS-TEOS composite hollow particles.

SEM and TEM imaging of the OTES-TSPA-TEOS capsules reveal a shell thickness <30nm and a mean diameter of 2580 nm. Due to the thickness and elasticity of the shell, the capsules were seen to collapse with high-vacuum electron microscopy. The TEOS-MPTMS-TEOS capsules failed to collapse due a thicker, less elastic shell. A mean diameter of 2.58±0.13µm and a shell thickness of 97.3±3.4 nm were recorded. Zeta sizing of these capsules shows a hydrodynamic diameter of 3.06µm, and elemental composition obtained by Energy Dispersive X-ray Spectroscopy (EDXS) confirmed the presence of C, O, Si, S consistent with the organosilanes used in the shell formation.

(iv) Gas-Filled Microcapsules

For the 30nm OTES-TSPA-TEOS capsules, the thin and elastic shell collapsed under vacuum. Thus, to remove the solvent and fill the capsule with air, the THF was exchanged for low-boiling point dichloromethane (DCM), which could then be removed by introducing the capsules into water ~40°C to evaporate the solvent. In contrast, the stiffer shelled (100nm) TEOS-MPTMS-TEOS capsules were dried for 24-48 hours under vacuum, stored in air, and resuspended in water.

3.2 Ultrabubbles : Ultrasound Contrast Agents

To evaluate the acoustic properties of OTES-TSPA-TEOS 'Ultrabubbles', the backscatter signal intensity was recorded as a function of mechanical index (M.I).

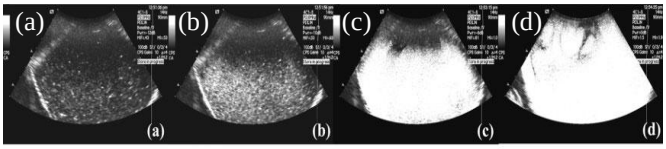

Figure 5 Ultrasound images of OTES-TSPA-TEOS capsules. The gain was kept at 10dB with increasing MI, MI = (a) 0.53, b) 0.73, c) 0.9, and d) 1.5.

The non-linear backscatter signal was found to increase along the focus band as a function of increasing M.I, thus confirming the pressure dependency of the backscatter. At the concentration of capsules used (5 x 10^8/ L) a signal comparable to that of the lipid-based UCA's was produced, however the commercial lipid UCA's (Sonovue) show sufficient backscatter between 0.07 to 0.5 M.I. whilst the OTES-TSPA-TEOS capsules have an imaging threshold ~ M.I 0.5, the acoustic pressure could be increased to M.I. ~1.9, without a significant loss of capsules due to inertial cavitation. Thus the OTES-TSPA-TEOS UCA's are more stable at much larger acoustic pressures and remain stable months after preparation enabling a longer shelf-life. Furthemore, the high glass transition temperature, Tg of the organosilica/silica matrix should enable heat sterilization of the UCA'a, eliminating potential immunogenicity issues. The different behaviour of the silica based capsules compared with the lipids can be expected by comparison of the material properties of the 'liquid' lipid shell in contrast to the more rigid organosilica/silica matrix. Thickness of the stabilising shell also influences the response; the lipid based UCA's generally produce a shell between 5-10nm (depending on lipid) compared with the 20-30nm designed for the OTES-TSPA-TEOS capsules. While stabilising the 'bubble', the presence of a shell impedes the oscillation. These microbubbles offer a very versatile alternative to the protein and lipid based UCA's currently available and can

be tailored to tune the acoustic pressure sensitivity according to application.

3.3 Ultrabubbles : Ultrasound Controlled Rupture

Whilst the OTES-TSPA-TEOS microbubbles, were designed to be resistant to inertial cavitation through the development of a highly elastic organosilica/silica shell, microbubbles for drug delivery are designed to resist inertial cavitation within a defined M.I range, but induce controlled rupture above a certain M.I. TEOS microbubbles rupture or crack without showing non-linear backscatter, but a capsule with a combination of MPTMS and TEOS can be tuned to generate a non-linear backscatter signal which increases as a function of M.I, whilst rupturing at a specific acoustic threshold pressure, potentially providing a future mechanism for drug delivery.

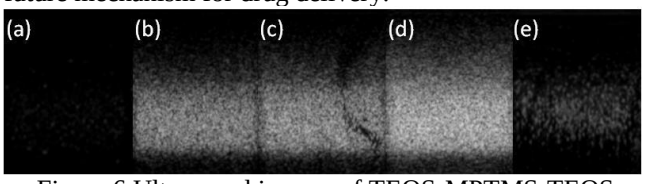

Figure 6 Ultrasound images of TEOS-MPTMS-TEOS capsules. The gain was kept at 10Db with increasing M.I, MI = a) 0.07, b) 0.44, c) 0.52, d) 0.8 and e) 1.2.

As recorded with the OTES-TSPA-TEOS capsules (figure 6), an increase in the non-linear backscatter as a function of increasing M.I between 0.07 to 0.8 is observed. Above 0.8, a low contrast image is recorded by the ultrasound scanner, confirming the acoustic pressure-selective irreversible rupture of the capsule. Conversion of the images into grey-scale data further confirms this controlled rupture (Fig 7). A near linear increase in the acoustic backscatter is recorded as a function of M.I, until M.I = 1.2 where a significant reduction is recorded. Within 1 second of insonation 82% of the capsules are ruptured. At higher acoustic pressures, or length of exposure 100% of the capsules rupture. Comparing the OTES-TSPA-TEOS and MPTMS-TEOS, the capsules with higher TEOS content are expected to show increased stiffness and brittleness, supporting the outline design philosophy for a suitable acoustic-sensitive drug delivery vehicle.

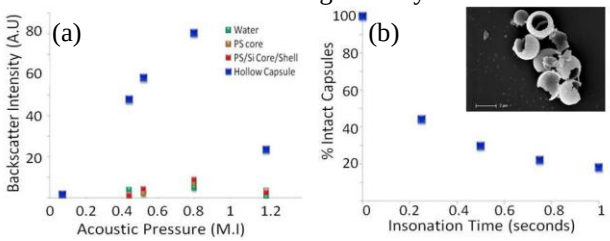

Figure 7 Grey scale image processing of the recorded ultrasound images, a) The ultrasound backscatter intensity as a function of mechanical index with solid particle controls and b) the intensity of the ultrasound backscatter of capsules exposed to M.I = 1.2 as a function of time (inset) SEM micrograph of ruptured capsules.

4 CONCLUSIONS

A versatile, tunable family of silica based capsules have been developed of varying diameter, shell thickness and shell properties such as elasticity and hydrophobicity. The tunability of these capsules is demonstrated in ultrasound applications. Their use as ultrasound contrast agents has been explored and preliminary results presented for their potential for controlled release systems. Future work will further examine the acoustic properties of the capsules and explore how these are affected by increasing concentration of an encapsulant, and its controlled release following insonation.

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