Multicore expandable microbubbles: Controlling density and expansion temperature

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Abstract

Stable microbubbles can be prepared by evaporation of a liquid core inside an expandable polymeric shell. To control the expansion temperature and the size of resulting microbubbles, we prepared polymeric microcapsules that contain multiple liquids in their core. One-step suspension polymerization allowed for encapsulation of at least two different liquids with near quantitative yield. The liquids may be miscible or immiscible, which directly affects the vapor pressure inside the capsule according to either Raoult's or Dalton's law, respectively. In the case of miscible liquids, e.g., perfluorohexane and perfluoropentane, both the vapor pressure and the expansion temperature vary within a range bounded by the properties of the neat liquids. For immiscible liquids, e.g. perfluoropentane and isopentane, the total vapor pressure is above this range as it equals to a sum of the individual pressures. Due to increased vapor pressure, encapsulation of two immiscible liquids significantly lowers the expansion temperature below the corresponding temperatures observed during expansion of microcapsules with neat perfluoropentane and isopentane cores. The microbubble diameter and shell thickness were controlled within ca. 10-50 μm and 0.1-3 μm , respectively, by varying the fractions and vapor pressure of the core fluids. We also showed that the effect of core composition on the expansion temperature was more significant than the effect of the shell thickness. Furthermore, one of the encapsulated materials may carry additional functions including imaging contrast, catalysis, and density compensation. To that end, these designer microcapsules may find applications in the fields of drug delivery, acoustic imaging, drilling, and self-healing materials.

Introduction

Polymer-shelled microcapsules (PSMs) display different applications depending on the encapsulated payload. Liquid-filled PSMs deliver drugs, lubricants, radiographic contrast agents, and reagents that can be released upon changes in pH, temperature, mechanical stress, and application of ultrasound.[1-13] Gas-filled PSMs can be used as density attenuators and acoustic contrast agents due to their high compressibility.[14-20] A particularly useful system is PSMs containing a gas-liquid-solid mixture (Figure 1a), which allows for multifunctional applications, such as acoustic navigation of chemicals delivery and dual imaging by acoustic and X-ray techniques.[21-26] Such microcapsules have been recently prepared using microfluidic processes[27-29] and by a multistep process involving solvent evaporation, freeze drying, and fractionation.[6] Another widely used technique for preparation of gas-liquid microcapsules is based on encapsulation of a volatile liquid (a blowing agent) followed by its evaporation at an elevated temperature.[30-33] Subsequent cooling below the glass transition of the polymeric shell ensures quenching of expanded microbubbles filled with saturated vapors of the blowing agent. As shown in Figure 1, this process allows for significant expansion and thinning of a polymeric shell that depend on the volume fraction of the blowing agent.

Figure 1. (a) Microcapsule with a multicomponent core, including liquid 1 (e.g., blowing agent), liquid 2 (e.g., drug), which may be miscible or immiscible with liquid 1, saturated vapors of liquids 1 and 2, and a solid fraction (3), all confined within a polymeric shell (4). Electron microscopy micrographs show microcapsules before (b) and after (c) expansion. Expansion occurs when a microcapsule (b.i) containing

a liquid blowing agent is heated above the boiling temperature of the core liquid T_{bp} and the glass transition temperature of the shell material T_g , inflating it into a microbubble (c.i) filled with a vapor of the blowing agent (gas). Photography of capsules in water-filled vials (b.ii, c.ii) shows decrease in density for expanded microbubbles. SEM at equal scale (b.iii, c.iii) of 100 vol% PFH composition shows size increase of 14 μm for unexpanded capsules to 46 μm for expanded bubbles. SEM of FIB milled capsules (b.iv, c.iv) reveal the decrease in shell thickness from 3 μm in unexpanded to 80 nm in expanded capsules (100 vol% PFH formulation).

One of the outstanding challenges in this technology is controlling the temperature of expansion and dimensions of expanded microbubbles. The dimensions include radius and shell thickness – two key parameters that determine vital properties of microbubbles, including compressibility, buckling stability, echogenicity, and release rate of an encapsulated material. We have addressed these two issues by encapsulating multiple (at least two) liquids followed by expansion of either one or both liquids inside the capsule. The expansion process depends on miscibility of the encapsulated liquids, which determines the vapor pressure and hence the onset temperature of the expansion process. We have shown that by encapsulating different types of liquids (miscible and immiscible) and controlling their molar fractions inside the core, we can adjust both the expansion temperature and expansion ratio within broad ranges, i.e. $T_{exp} = 90 - 190^\circ\text{C}$ and $V/V_0 = 1 - 30$, respectively. One fundamental challenge addressed in this paper is to demonstrate that two chemically different liquids (especially, immiscible ones) can be encapsulated within a single core at a stoichiometric molar ratio defined by the composition of the macroscopic mixture. These multicomponent microcapsules have been prepared using a simple, scalable, one-step suspension polymerization technique with near quantitative yield allowing multiple components for encapsulation. Unlike previous studies where the expansion temperature was controlled by changing the mechanical properties of the shell,[33,34] we tune the expansion behavior by varying composition of a blowing agent. These microcapsules represent a general system that could perform multiple, simultaneous functions for a number of applications. For example, if the cargo is a drug, then upon expansion, polymeric microcapsules containing a gas and a liquid drug are formed. This is similar to, but more stable than lipid stabilized microcapsules developed by other groups.[35-39] Such microcapsules could perform ultrasound-triggered drug release while also enhancing acoustic imaging contrast. Another possible application of these particles is for self-healing materials. It can require a large amount of force to rupture a microcapsule with an incompressible liquid core. To solve this problem, researchers have developed hollow particles that are vacuum infiltrated to produce a particle that

is only partially filled with liquid.[38] However, this process requires several steps and has, at each step, a portion of unsuccessful microcapsules that are removed. Thermal expansion of a microcapsule containing a liquid for self-healing could represent an easier route to partially filled microcapsules of desired composition.

Experimental

Materials. Acrylonitrile (AN, 99%+, stabilized with 40 ppm monomethyl ether hydroquinone), methacrylonitrile (MAN, 99%, stabilized with 50 ppm monomethyl ether hydroquinone), *n*-decane (99%+), poly(vinyl pyrrolidone) (PVP, average M.W. 10000) were purchased from Acros Organics. 1,1-dichloroethylene (VDC, stabilized with 200 ppm monomethyl ether hydroquinone as inhibitor), 2,2'-azobis(2-methylpropionitrile) (AIBN, 98%), acrylic acid (AA, 99%, stabilized with 180-200 ppm monomethyl ether hydroquinone), Oil Red O (ORO, certified grade), isopentane (IP, 99%), perfluorohexane (PFH, 99%), perfluoropentane (PFP, 99%), perfluorooctane (PFO, 99%), trimethylolpropane triacrylate (TMPTA, stabilized with 100 ppm monomethyl ether hydroquinone), tetramethylsilane (TMS, NMR grade 99.9%+), sodium 2-ethylhexylsulfate (~50% in water), LUDOX HS-30 colloidal silica (30% suspension in water), glacial acetic acid were purchased from Sigma Aldrich. Sodium hydroxide (NaOH, 95%+), magnesium chloride hexahydrate ($\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 99%+), potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$, 99%+) were purchased from Fisher Scientific. Monomethyl ether hydroquinone (MEHQ) inhibitor was removed by passing through a column of basic aluminum oxide (Sigma Aldrich, activated, basic, Brockmann I).

Polymerization. Oil/water emulsions were stabilized by either magnesium hydroxide ($\text{Mg}(\text{OH})_2$) colloid particles or colloidal silica particles, depending on the type of shell monomers used. In a typical run, an oil phase was prepared in a 4 mL Vialmix® vial consisting of AIBN (0.010 g), AN (0.210 g), 10 wt% TMPTA in AN solution (0.030 g), VDC (0.826 g), and 0.418 mL of core liquid mixture of PFH and *n*-decane (dyed with ORO of 5.33×10^{-3} M). Table 1 outlines different PFH/*n*-decane ratios used in this study. A water phase was prepared in a 25 mL scintillation vial consisting of deionized water (12.86 g), NaOH (0.244 g), and $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ (0.857 g). This mixture was stirred vigorously for 30 minutes, degassed, and 0.046 g of a 1 wt% aqueous solution of sodium 2-ethylhexyl sulfate was added. The water phase (2.12 g) was added to the organic phase and the Vialmix ® vial was sealed with a septa and cap using

the Wheaton® E-Z crimperTM and shaken three successive times on the Vialmix® to create a suspension. The suspension was then placed in the oven at 63 °C to polymerize for 16 hours. Prior to expansion tests, the product was passed through a 25 µm sieve, washed with 10 wt% acetic acid to remove Mg(OH)₂ colloidal stabilizer, collected by vacuum filtration, and dried at room temperature for further analysis.

Table 1. Poly(AN-c-VDC) microcapsule formulation¹

Shell monomers ²			Core components ⁴			AIBN ⁵ (g)	Water ⁶ (g)
AN (g)	10 wt% TMPTA ³ in AN (g)	VDC (g)	PFH/ <i>n</i> - decane (v/v%)	<i>n</i> -decane (g)	PFH (g)		
0.105	0.015	0.413	100/0	0.0000	0.3487	0.005	1.06
0.105	0.015	0.413	50/50	0.0763	0.1743	0.005	1.06
0.105	0.015	0.413	10/90	0.1372	0.0349	0.005	1.06
0.105	0.015	0.413	5/95	0.1449	0.0174	0.005	1.06
0.105	0.015	0.413	1/99	0.1510	0.0035	0.005	1.06
0.105	0.015	0.413	0/100	0.1525	0.0000	0.005	1.06

¹The total volume of the oil phase (shell monomers) was kept constant of 0.696 mL, while the shell/core volume fraction was varied from 90:10 to 30:70 (v/v%). ²AN/VDC ratio was varied from 0/100 to 100/0 (v/v%), typically AN/VDC=30/70 was used. ³Crosslinking density was varied from 0 to 2.2 wt%, typically 0.28 wt% was used. ⁴Volume fraction of the blowing agent (PFH) in a core was varied from 0 to 100 vol%. ⁵[AIBN] = 1 wt% of monomers (g). ⁶Water phase = monomers (g) × 2

In a different set of microemulsions, we used water-soluble AA as shell monomer. In this case, colloidal silica particles were used as a stabilizer along with PVP and K₂Cr₂O₇ (inhibits polymerization in the water phase), while the oil phase consisted of AIBN (0.006 g), AN (0.442 g), 1 wt% TMPTA in AN solution (0.01 g), AA (0.162 g) and 0.3 mL of core liquid components (certain ratio of PFH/*n*-decane) was mixed with a water phase consisted of LUDOX HS-30 (0.03 g), 30 wt% PVP aqueous solution (0.02 g), 25 wt% K₂Cr₂O₇ aqueous solution (0.016 g) and deionized water (2 g). The pH of water phase was adjusted to 4 by hydrochloric acid. The mixture was degassed, sealed and shaken in the same way above. Polymerization was conducted at 63 °C for 16 hours.

Optical microscopy. To monitor the expansion process, liquid-filled microcapsules were deposited onto a glass slide and heated using a Mettler FP82HT hot stage at 6 °C/min. Optical micrographs were captured once every 0.5 °C with an 18.0 megapixel DSLR (Canon 650D) attached to an optical microscope (Zeiss Axioskop 2). Using Image-J software, frame-by-frame

automated measurement of capsule area allowed calculation of radius (R) and volume (V), assuming the capsules to be spherical. Expansion curves were constructed by plotting the ratio of the expanded volume to the original one (V/V_0) as a function of temperature. The expansion temperature (T_{exp}) was determined from the expansion curve as the temperature at which the V/V_0 ratio starts deviating from unity. T_{max} corresponds to the temperature in which maximum volumetric expansion occurred. The heating rate was varied in a broad range to ensure accuracy and reproducibility of the expansion curves (Figure S1 in Supporting Information)

SEM and FIB. Capsule size was determined by scanning electron microscopy (SEM) using the FEI Helios 600 Nanolab Dual Beam System. To determine shell thickness, focused ion beam (FIB) milling of particles was employed with gallium ions at 30 kV. A 10 nm gold-palladium coating was used to prevent charging and distortion of images.

Confocal Raman microscopy was performed on individual microcapsules at ambient conditions to verify chemical composition of their cores. Samples were imaged using a Renishaw inVia Raman microscope: excited with a 514 nm laser at 0.1-0.5 mW; passed through a 1800 lines/mm grating; magnified with 50 $\times$ objective; 5-10 s exposure time per scan; 1-3 scans were averaged for one image. The spectra of ten individual capsules were averaged to form a representative spectrum for each formulation.

Results and discussion

a) Microcapsules: Synthesis and characterization

Polymeric microcapsules were synthesized by suspension polymerization, wherein volatile compounds (hydrocarbon, perfluorocarbon, and organic silanes) were encapsulated inside a polymeric shell (Figure 1a). To enable expansion, microcapsules should have a relatively thin shell provided by a significant fraction of the liquid core.[31] As such, we have studied microcapsules with shell-to-core ratio ranging from 30:70 to 70:30 v/v%.

Shell. A suitable combination of mechanical, thermal, and gas-permeability properties is particularly vital for the design of expandable microcapsules. AN exhibits excellent gas-barrier properties, yet, relatively poor heat-resistance.[30-33] Therefore, copolymerization of AN with other monomers, such as VDC and MAN, was used in the shell material to ensure preparation of robust microbubbles. As shown in Figure 2(a-d), the variation of co-monomer molar ratio (AN/VDC) altered both the morphology and size of the produced microcapsules resulting in

heterogeneous shells over most of the composition range (e.g. 74/26 and 34/66 mol/mol%), while more homogeneous shells were observed only for the extreme compositions (e.g. 100/0 and 0/100 mol/mol%). The morphology variation is ascribed to shell expansion during synthesis at elevated temperatures. Vaporization of the blowing agent causes expansion of the AN/VDC microdroplets during polymerization, which is followed by shrinkage upon cooling resulting in heterogeneous look. On the contrary, the extreme compositions do not successfully inflate, leading to a more homogeneous morphology. Full analysis of correlations between the capsules structure and their expansion properties will be discussed in the next section. This composition-dependent behavior is consistent with the previous reports identifying two broad classes of polymerization systems: (i) polymers which swell in their own monomers, e.g., PMMA or PSt, form homogeneous bulk and surfaced morphologies, while (ii) polymers, which precipitate during polymerization, like PAN and PVDC, form heterogeneous and irregular-shaped morphologies.[41-44]

Figure 2. SEM images of poly(AN-c-VDC) capsules with different copolymer compositions: a) AN/VDC = 0/100, b) AN/VDC = 34/66, c) AN/VDC = 74/26, d) AN/VDC = 100/0 (mol/mol%). Shell:core=70:30 (v/v%), PFH/*n*-decane (30/70 v/v%). Scale bar 100 μ m.

Core. Microcapsules expand when vapor pressure of the core liquid reaches a critical expansion pressure to overcome mechanical resistance of the shell (see next section). We have employed two techniques to monitor the core composition inside individual microbubbles. First, ORO dye (which is soluble in *n*-decane and exhibits strong fluorescent emission around 600 nm) has been added to *n*-decane solution prior to emulsification with PFH. Using 3D fluorescence microscopy, we have verified successful encapsulation of the additional compound (Figure 3a). After the PFH expansion, the *n*-decane component was imaged as droplets attached to the internal wall of the polymeric shell (Figure 3b). Second, using confocal Raman microscopy, we have measured intensity of two distinct Raman bands at 761 cm^{-1} and 2840 cm^{-1} that correspond to PFH and *n*-decane, respectively (Figure 3c). For quantitative analysis of the liquid core composition, we

have measured the relative intensity of each liquid (PFH and *n*-decane) encapsulated with different fractions. The relative variation of the peak intensities is consistent with the corresponding compositions of the macroscopic mixtures prior to emulsification (Figure 3d).

Figure 3. Bright field and fluorescent optical micrographs visualize *n*-decane droplets (2) in the PFH core (1) inside a 34/66 mol/mol% poly(AN-c-VDC) polymeric shell (a) before and (b) after expansion due to PFH evaporation. (c) Confocal Raman spectra of individual polymeric microcapsules (before expansion) were measured for four different compositions of the core mixture as indicated. (d) Relative intensities of the Raman peaks (dashed ovals in c) for *n*-decane (circles) and PFH (squares) as a function of the volume fraction of PFH in the microcapsule core. For the further quantification of core compositions, the relationships between the concentration of each core component and the relative intensity have been derived: $I/I_0 = \phi$, where I corresponds to peak intensity for each liquid in various compositions of PFH/*n*-decane (0/100-100/0 vol/vol%) inside the microcapsules and I_0 corresponds to peak intensity for 100 vol% pure PFH or *n*-decane inside the microcapsules, and ϕ is the molar fraction of PFH or *n*-decane inside of the microcapsules. The error bars show variations of these relative intensities within each microcapsule subset that contain the same core composition.

b) Controlling the expansion temperature

Expansion of a microcapsule possessing a rubber-elastic shell occurs when the internal inflation pressure exceeds the sum of the external pressure and the restoring pressure caused by stress in the biaxially extended shell ($P_{in} > P_{ext} + P_{el}$). While P_{ext} is assumed to be constant, the restoring pressure in a linear elasticity regime increases with microcapsule expansion as:

$$P_{el} \cong 4G \frac{h_0}{R_0} \left(\frac{1}{\lambda} - \frac{1}{\lambda^7} \right) \quad (h_0 \ll R_0) \quad (1)$$

where $G \cong \nu kT$ is the shear modulus of a polymer network (shell material) with a crosslinking density ν , h_0 is the initial shell thickness, R_0 is the initial shell radius, and $\lambda = R/R_0 = \sqrt{h_0/h}$ is the shell extension ratio.[45] The variation of the restoring pressure is shown in Figure 4a. As the inflation pressure rises and a microcapsule expands, the restoring pressure increases until it reaches a maximum at $\lambda^* \cong 1.38$, where the shell becomes unstable and exhibit instantaneous expansion (horizontal dashed arrow) controlled by finite extensibility (λ^{**}) of the network strands. At this limit, the extension ratio is significantly larger than λ^* and may lead to plastic deformation and even shell rupture. The experimentally observed onset of microbubble expansion corresponds to maximum inflation pressure sustained by a rubber-elastic shell as:

$$P_{in}^* \cong P_{ext} + P_{el}(\lambda^*) \quad (2)$$

For a hypothetical shell ($h_0 = 1 \mu m$, $R_0 = 10 \mu m$, $G = 1 MPa$) and $P_{ext} = 1 atm$, equations 1 and 2 gives $P_{in}^* \cong 3 atm$. This estimation is approximate and varies depending on the shell dimensions, crosslink density, and temperature. More accurate P_{in}^* values for the microcapsules studied in this paper are summarized in Table 2.

Equation 2 indicates two ways to shift the onset of the expansion process. One conventional approach is based on controlling the restoring pressure (P_{el}), which depends on the mechanical properties of the shell determined by thickness and radius of the shell and modulus of the shell material. The second approach (main focus of this study) is based on controlling the inflation pressure (P_{in}), which is determined by vapor pressure of a blowing agent inside the core. As shown in Figure 4b, microcapsules with a PFP core demonstrate higher vapor pressure than that of a PFH core at the same temperature and, therefore, are expected to expand at a lower temperature.

Figure 4. (a) Initially, the restoring pressure P_{el} of an inflated rubber-elastic shell exhibits steady increase with expansion ratio $\lambda = R/R_0$ reaching maximum at $\lambda^* = 1.38$ (equation 1).[45] At $\lambda > \lambda^*$, the progressive shell thinning as λ^{-2} results in expansion instability (dashed arrow), which is halted by finite extensibility of the network strands (magenta diamonds). (b) Vapor pressure of PFP and PFH increase with temperature according to the Clapeyron-Clausius equation. The empty and solid circles correspond to the inflation pressure ($P_{in}^* = 8.5 \pm 0.5 \text{ atm}$) at the measured expansion temperatures of PFP ($T_{exp} = 106^\circ\text{C}$) and PFH ($T_{exp} = 137^\circ\text{C}$) as shown by long dashed lines, respectively. The solid squares correspond to vapor pressures calculated from equations 1 and 2 for three different h_0/R_0 ratios of the poly(AN-c-VDC) shells (Table 2). The intersections with the PFH vapor pressure (red line) predict T_{exp} for the corresponding shells (short dashed lines).

Effect of the shell structure. We first present the effect of shell dimensions on the expansion temperature. Specifically, we have prepared microcapsules with different h_0/R_0 ratios, where h_0 - shell thickness and R_0 - shell radius prior to expansion. The size and thickness of the microcapsules are controlled by volume ratio of shell monomers to core liquids in the synthetic formulation, while maintaining the same chemical composition and crosslinking density of the shell material. Formulations with a larger shell-to-core ratio yield capsules with a thicker shell

and lead to a higher T_{exp} . The dimensions and expansion properties of poly(AN-c-VDC) capsules with different shell-to-core ratio are summarized in Table 2. As expected, a decrease in the h_0/R_0 ratio (thinner shells) results in the corresponding decrease of the T_{exp} . The measured T_{exp} values demonstrate good agreement with the corresponding values predicted by equation 2 and depicted by short dashed lines in Figure 4b. For this analysis, we used a constant shear modulus of $G = 2.3 \pm 0.2 \text{ MPa}$ measured separately at $T = 400 \text{ K}$. The assumption of a constant modulus is valid because the $\sim 30 \text{ K}$ temperature variations around 400 K correspond to less than 10% modulus shifts, which is trivial on the logarithmic scale.

To further verify the effect of the shell structure, we replaced VDC with AA in the shell composition, which is known to provide superior heat resistance.[34] Similar to the poly(AN-c-VDC) samples, the shell thickness was controlled by varying the shell-to-core volume fraction in the microemulsion formulation. As expected, the more rigid AN/AA capsules ($G = 2.9 \pm 0.2 \text{ MPa}$ at $T = 400 \text{ K}$) led to a higher expansion temperature compared to the AN/VDC composition (Table 2). However, the variation of the h_0/R_0 ratio displayed the same trend, i.e. the expansion temperature decreases with decreasing h_0/R_0 .

To summarize, controlling the shell thickness and shell rigidity allowed for consistent shifts in the expansion temperature. However, these variations were relatively moderate compared to the effect of the core composition discussed below.

Table 2. Correlation between dimensions and expansion temperatures for poly(AN-c-VDC) and poly(AN-c-AA) microcapsules.

Composition ¹	34/66 mol/mol% P(AN-c-VDC)			79/21 mol/mol% P(AN-c-AA)		
Shell:core ²	70:30	50:50	30:70	70:30	50:50	30:70
R_0 (μm)	3.6	3.3	3.3	4.9	6.5	6.8
h_0 (μm) ³	0.80	0.57	0.50	1.2	0.85	0.51
h_0/R_0	0.22	0.17	0.15	0.24	0.13	0.08
P_{in}^* (atm) ⁴	13.5	10.6	9.5	18.2	9.3	5.8
Predicted T_{exp} ($^{\circ}\text{C}$) ⁵	159	147	143	181	140	118
Measured T_{exp} ($^{\circ}\text{C}$) ⁶	150	140	137	175	165	150

¹) All compositions contain PFH/*n*-decane (50/50 v/v%) and 0.28 wt% crosslinker. ²) Shell:core ratio varies from 70:30 to 30:70 by volume. ³) Shell thickness measured by sectioning microcapsules with SEM-FIB.

⁴) Maximum inflation pressure is calculated using equation 2 at $\lambda^* \cong 1.38$ (shell extensibility at the maximum restoring pressure) and $P_{ext} = 1 \text{ atm}$. ⁵) Temperature at which the vapor pressure of perfluorohexane equals to P_{in}^* (equation 2) as indicated by short dashed lines in Figure 4b. ⁶) Expansion temperatures measured when 80% of microcapsules expanded.

Effect of the core composition. We first studied the effect of neat liquids, i.e. single component cores. Different blowing agents (PFH, PFP, PFO, IP, decane, hexane, TMS) were incorporated into the same poly(AN-c-VDC) shell. It was found that the triggering temperature for expansion process strongly depended on the core composition. Figure 5a compares different blowing agents composed of different fractions of PFP and PFH. The single component cores (100% PFP and 100% PFH) show significantly different expansion temperatures $T_{exp} = 106^{\circ}\text{C}$ and $T_{exp} = 137^{\circ}\text{C}$, respectively. These temperature values correspond to the same vapor pressure $P_{in}^* = 8.5 \pm 0.5 \text{ atm}$ of the neat PFP and PFH given by the Clapeyron-Clausius equation (Figure 4b). In other words, both PFP- and PFH-filled microcapsules at 106°C and 137°C , respectively, reach approximately the same inflation pressure $P_{in}^* = 8.5 \pm 0.5 \text{ atm}$, as required for expansion of microcapsules with the same shell structure. Note that all T_{exp} 's are significantly above the glass transition temperature of the shell material ($T_g = 80^{\circ}\text{C}$) and also above the boiling temperatures of the core liquids.

Single component cores provide a limited range of expansion temperatures. To expand the range and enable fine shifting of T_{exp} , we prepared microcapsules with two liquids inside the core. And, we studied two distinct systems: microcapsules loaded with (i) miscible and (ii) immiscible liquids.

Miscible liquids. For a mixture of two miscible fluids, such as PFP and PFH, the vapor pressure follows the Raoult's law, which states that the total vapor pressure of an ideal solution is the molar average of the vapor pressures of the constituting molecular components. In this case, we expect seeing T_{exp} ranging between the corresponding temperatures of the neat liquids. As shown in Figure 5a, the expansion temperature exhibits consistent variations within a range of 106°C to 137°C upon changing the PFP:PFH composition, while maintaining the same shell structure. However, the resulting T_{exp} remains confined within boundaries controlled by the expansion temperatures of the neat liquids.

Figure 5. Experimentally measure expansion curves of the same poly(AN-c-VDC) shell filled with multicomponent cores including a) miscible (PFH and PFP) and b) immiscible (IP and PFP) components. In agreement with Figure 4b, PFP-filled microcapsules start inflating at a lower temperature of $T_{exp} = 106^{\circ}\text{C}$, while PFH with a lower vapor pressure initiates expansion at a higher temperature of $T_{exp} = 137^{\circ}\text{C}$. Yet, both temperatures correspond to nearly the same vapor pressure $P_{in}^* = 8.5 \pm 0.5 \text{ atm}$ as expected for the same shell composition (equation 2). For the immiscible liquids mixture, the total vapor pressure is a sum of that of the pure liquid, thus, T_{exp} shifts to lower temperature. The difference in the maximum V/V_0 between microcapsules with different core compositions is due to ill-defined variations of core fraction during encapsulation.

Immiscible liquids. To enable expansion at lower temperatures, we have prepared microcapsules encapsulating two immiscible liquids, e.g. PFP and IP. In this case, the vapor pressure of the mixture obeys the Dalton's law: $P_{mix} = P_A^{\circ} + P_B^{\circ}$, where P_A° and P_B° are the vapor pressures of pure A and pure B. In other words, the mixture vapor pressure is always greater than the vapor pressure of the pure components. Moreover, it does not depend on the molar fractions of the individual components. This suggests that T_{exp} of a mixture will always be lower than that of microcapsules loaded with any of the individual liquids. Our experiments have corroborated this prediction. As shown in Figure 5b, the expansion temperature of these capsules is below that of capsules with the neat IP and PFP. And, in contrast to the miscible systems, the expansion temperature does not depend on the composition.

c) Control of expansion ratio

The amount of encapsulated blowing agent is also directly related to the expansion ratio and resultant decrease in the shell thickness. Hence, the concept of immiscible components was

implemented to control the expansion ratio of the capsules. For the immiscible system, a mixture of *n*-decane and PFH (the blowing agent) was studied. Figure 6a shows expansion of poly(AN-c-VDC) microcapsules with different volume fractions of PFH. The optical images (Figure 6a) clearly illustrate the changes in expandability by varying the PFH content from 5 vol% to 100 vol%, which clearly indicates that PFH and *n*-decane are co-encapsulated during the polymerization. In conjunction with the previous results, the expansion process starts at a temperature of 137°C, which is above both the boiling temperature of PFH ($T_{bp} = 56^\circ\text{C}$) and the glass transition temperature of the shell material $T_g = 80^\circ\text{C}$. [33,34] As discussed below, the higher temperature generates sufficient vapor pressure needed to overcome the elasticity of the shell. Another important experimental observation is that the expansion temperature does not depend on the loading of *n*-decane (Figure 6b). This loading ratio independence follows directly from Dalton's law, where the vapor pressure is the same for a given temperature regardless of the molarity of the components.

Since one of the encapsulated liquids, *n*-decane, does not boil at the pressures and temperatures considered, we can control the expansion ratio by varying the molar fraction of PFH in the core. Figure 6c combines two log-log plots corresponding to volume expansion and shell thinning with increasing mass fraction of PFH. The volumetric expansion ratio (V/V_0) increases linearly with the fraction of the blowing agent up to 6 vol%. At higher fractions, the expansion ratio deviates from linear dependence due to finite extensibility of the shell and gas effusion. The second set of data points in Figure 6c corresponds to average shell thickness determined by FIB sectioning of individual microbubbles. A power-law fit follows $h \sim \phi^{-0.56 \pm 0.12}$, which demonstrates a good agreement with the theoretical $\phi^{-2/3}$.

Figure 6. Variation of the blowing agent (PFH) content, ϕ and its effect on expandable properties of the poly(AN-c-VDC) microcapsules; a) Optical and SEM images of expanded microbubbles containing different fraction of PFH in the core as indicated (The same scale bar is applicable to all images); b) expansion curves as a function of ϕ ; c) dependence of maximum expansion and shell thickness on ϕ .

Conclusion

We have demonstrated a successful one-step synthesis of microcapsules allowing co-encapsulation of two chemically different liquids. These microcapsules exhibit drastic volume change by thermally triggered expansion when the restoring pressure of the polymeric shell is overcome by the increase in internal vapor pressure at the critical expansion pressure. The control of T_{exp} , in the range between 90 and 190°C , was implemented through variation of thermo-mechanical stability of the shells, i.e., the shell thickness, glass transition temperature. Furthermore, through variation of the blowing agents in the microcapsules core, we are able to control the critical vapor pressure required for the expansion, resulting in different T_{exp} for an identical shell. The variation of the blowing agent fractions, also enabled us to control the expansion ratio in a range of $V/V_0 = 1\text{--}30$. Correspondingly, this allows for controlling the shell thickness and compressibility of polymeric shells. By reducing the volume fraction of the blowing agent (therefore increasing the fraction of the non-volatile component) we can change

the expansion ratio of the microbubble. The concurrent activation and complementarity enables a broad range of practical applications, such as contrast agents, delivery vehicles, and density attenuators. The inclusion of a second liquid, in addition to the blowing agent, dramatically expands the range of applications of gas-filled polymeric microcapsules. While the blowing agent decreases density, increases size, and enhances echogenicity, the second material carries complementary valuable functions such as imaging contrast and catalysis, all while its relative abundance tunes the expansion ratio. We showed that only a small amount of blowing agent (~1 vol%) is required to cause thermal expansion of microcapsules containing up to 99 vol% of non-volatile cargo, provided that it is immiscible with the blowing agents. Similarly, incorporation of a small amount of immiscible blowing agent can be used to lower the T_{exp} of existing microcapsule formulations, which may be useful in research and industry applications where low T_{exp} is desirable.

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Expandable shells