

Monodisperse Stimuli-Responsive Colloidosomes by Self-Assembly of Microgels in Droplets

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We introduce a novel and versatile technique to fabricate monodisperse stimuli-responsive colloidosomes using stimuli-responsive microgel particles as building blocks, aqueous droplets as templates, and microfluidic devices to control the assembly. Our colloidosomes exhibit ~80% decrease in volume when actuated; thus, they can be of immense potential in applications that require targeted pulsed-release of active materials. The use of microfluidics allows fabrication of extremely monodisperse colloidosomes. Alternatively, our technique can also be combined with bulk emulsification techniques to produce large quantities of colloidosomes for various applications.

Colloidosomes are hollow microcapsules with a shell composed of densely packed colloidal particles.^{1–4} Their physical and chemical properties such as mechanical strength and biocompatibility can be tuned by selecting colloidal particles with desired rigidity and surface functionalities.^{5–8} The hollow structure of colloidosomes endows them with an ability to encapsulate materials, which can be subsequently released in a controlled fashion. This property makes colloidosomes promising vehicles for macromolecular delivery in pharmaceutical, cosmetics, and food industries.^{9,10} The primary mode by which materials are released from colloidosomes is diffusion through the interstitial pores between the colloidal particles. Sizes of these interstitial pores are determined by the size and polydispersity of the colloidal particles. Thus, by controlling these parameters, the rate of diffusion through colloidosomes can be finely tuned.^{3,11} However, diffusion by itself is an inadequate mechanism for applications that require bulk release of materials in a short time. Such applications necessitate engineering an additional material-release mechanism within colloidosomes. Incorporation of such a mechanism, which can preferably be triggered by an external stimulus, would further enhance the properties of colloidosomes and expand their application portfolio.

In this Letter, we present a novel technique to fabricate colloidosomes that exhibit a large change in volume when actuated by an external stimulus. Our colloidosomes are composed of

poly(*N*-isopropylacrylamide) (PNIPAm) microgels, which are thermosensitive cross-linked polymeric particles. When dispersed in water, these microgels undergo a rapid transition in size when the temperature is raised above the phase-transition temperature of the polymer (~32 °C).^{12,13} We take advantage of the self-assembly of these microgels in droplets to fabricate colloidosomes which can be shrunk or swollen reversibly by changing the temperature. We employ microfluidic techniques to ensure low polydispersity of the formed colloidosomes. Such monodisperse stimuli-sensitive colloidosomes can be of immense potential in applications that require targeted pulsed-release of active materials.

We begin with *N*-isopropylacrylamide monomer, NIPAm, which is polymerized along with a cross-linker by precipitation polymerization to yield an aqueous suspension of ~750 nm diameter PNIPAm microgel particles. Allylamine (~10 mol %) is copolymerized along with NIPAm monomer to incorporate reactive amine groups into and onto the microgel particles. The cationic nature of the microgels was confirmed by measuring the electrophoretic mobility of the particles which was found to be $\sim 1.35 \times 10^{-8} \text{ m}^2 \text{ V}^{-1} \text{ s}^{-1}$. The aqueous microgel suspension is emulsified in a silicon oil containing a surfactant. Right before emulsification, 1 vol % glutaric dialdehyde (glutaraldehyde) is added to the aqueous mixture. Once emulsified, the microgels self-assemble at the oil–water interface due to the presence of hydrophilic acrylamide groups and hydrophobic isopropyl groups.⁴ Glutaraldehyde molecules, owing to their two reactive sites each, serve as connecting links between the amine-functionalized microgels through an amine–aldehyde condensation reaction.^{14,15} Interlinking of the microgels at the oil–water interface results in the formation of colloidosomes. These colloidosomes are washed with isopropanol to remove silicon oil and are redispersed in water. The overall scheme is shown in Figure 1.

Any emulsification technique can be employed to emulsify the aqueous microgel suspension in oil. However, since our colloidosomes are formed using emulsified droplets as templates, their

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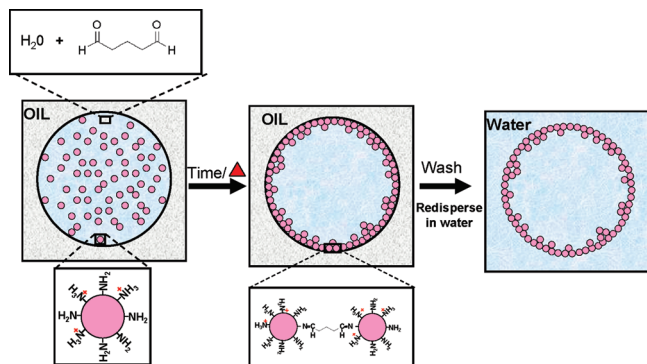


Figure 1. Schematic representation of the technique used for fabricating colloidosomes using poly(*N*-isopropylacrylamide) microgel particles as building blocks and emulsion droplets as templates. Aqueous droplets containing amine-functionalized PNIPAm microgels and glutaraldehyde are formed in silicon oil. Microgels assemble at the water–oil interface due to the presence of the hydrophilic acrylamide groups and hydrophobic isopropyl groups. The assembled microgels are linked together by glutaraldehyde through an amine–aldehyde condensation reaction. Colloidosomes with interlinked microgels are then washed and redispersed in water.

polydispersity is set by the polydispersity of the droplets. Size and polydispersity are important properties of colloidosomes, since these properties determine the amount of encapsulated material and its release kinetics. To fabricate colloidosomes that each encapsulate and release identical amounts of materials, we use emulsion droplets with very low polydispersity as templates; this is accomplished by employing microfluidic techniques to emulsify the microgel suspension. Drops are formed in microfluidic devices as a result of the balance of interfacial tension between the two fluids and shear exerted by the continuous fluid on the dispersed fluid. Since interfacial tension between the two fluids is constant and shear rate can be finely tuned in these devices, droplets with less than 1% polydispersity can be efficiently produced.^{16–20} Here, we demonstrate fabrication of monodisperse PNIPAm colloidosomes using a capillary-based microfluidic device. Our device is composed of two coaxially assembled glass capillaries, one with a square cross section and other with a round cross section. Coaxial assembly is achieved by choosing dimensions of the capillaries such that the outside diameter of the round capillary matches the inside dimensions of the square capillary. Before assembling, the round capillary is pulled with a pipet-puller to create a gradual taper that culminates in a fine orifice. The continuous-phase fluid, surfactant containing silicon oil, and the dispersed-phase fluid, aqueous microgel suspension containing glutaraldehyde, flow in from opposite ends of the square capillary. The oil phase focuses the aqueous phase into the tapered end of the round capillary. The aqueous phase breaks into monodisperse droplets upon entering the round capillary and the emulsion is collected at the other end of the round capillary as shown in Figure 2a.

Once collected, the emulsion is stored at room temperature. When these emulsion droplets are allowed to dry on a glass slide,

their surface gradually develops a crumpled morphology upon evaporation of water. This indicates that the droplets have a membranous shell which buckles as the droplet volume decreases upon evaporation of water from within (Figure 2b). We confirm the core–shell architecture of the droplets using confocal microscopy. Our analysis reveals self-assembly of fluorescently tagged microgels along the oil–water interface as shown in Figure 2c. Low intensity fluorescence within droplets suggests the presence of excess microgels in the bulk of the droplets. Upon reducing microgel concentration in the aqueous phase to ~10% of the original concentration, fluorescence intensity in the bulk of the droplets is reduced significantly without much change in the fluorescence intensity of the shell as shown in the inset in Figure 2c and in Figure 2d.

To ensure that colloidosomes thus formed maintain their structural integrity when redispersed in other fluids, it is necessary to lock the microgels in this conformation. Glutaraldehyde was added to the aqueous microgel suspension prior to emulsification to accomplish this objective. The two reactive aldehyde groups of glutaraldehyde can covalently bind to amine groups on two different microgels. We heat the emulsion for 30 min at 60 °C and then set it aside at room temperature for 48–72 h. The colloidosomes, with covalently interlinked microgels, are then repeatedly washed with isopropanol to remove any adsorbed silicon oil and thereafter washed and redispersed in water. The extended time prior to isopropanol washes is necessary to allow the linking process to be completed. Washing the colloidosomes with isopropanol immediately after heating the emulsion results in failure of the colloidosome structure due to “unzipping” of the microgels.

The morphology and microstructure of the colloidosomes were investigated using optical, confocal, and electron microscopy. The optical micrograph shows a spherical colloidosome with a soft deformable shell evident from the inward bending of the shell (Figure 3a). A confocal image of colloidosomes submerged in water confirms their core–shell architecture. The colloidosomes appear to have a shell of nonuniform thickness (Figure 3b). We believe this to be the combined effect of irregular packing of microgels in the shell and nonuniform linking of microgels by glutaraldehyde. It is conceivable that there is some variation in the number of glutaraldehyde molecules reacting with each PNIPAm microgel, which could lead to variations in interlinking capabilities of the microgels; also, it is possible that the two aldehyde groups of a glutaraldehyde molecule may react with amine groups on the same microgel, resulting in no interlinking. Such events could lead to formation of shells with uneven thickness. The three-dimensional structure of the colloidosomes is displayed by a movie created by stacking a series of planar confocal images taken along the *z*-direction (Supporting Information, Movie S1). The movie reveals colloidosomes with a flexible shell and a large internal core ($\sim 1.1 \times 10^5 \mu\text{m}^3$) that can be used to encapsulate materials for pulsed-release. Scanning electron microscope images of the colloidosomes show the ordering and packing of microgels in the shell. Regions of hexagonally closed-packed microgels are surrounded by randomly packed microgels as shown in Figure 3c.

We examine the thermosensitive behavior of the colloidosomes using a thermal stage mounted on a microscope. Colloidosomes are submerged in deionized water in a transparent sample holder which is placed atop the thermal stage. The equilibrium thermal response of the colloidosomes is determined by heating samples from 20 to 55 °C in fixed increments of 2 °C and then lowering the sample temperature back to 20 °C at the same rate. Samples are allowed to equilibrate at each temperature for 30 min before

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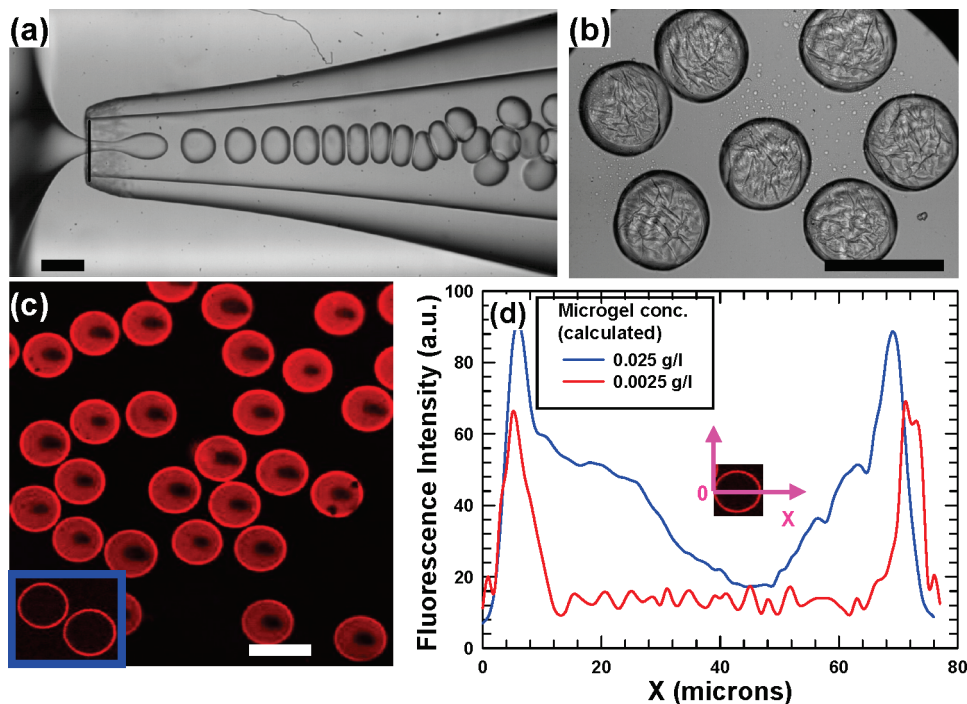


Figure 2. (a) Generation of monodisperse droplets in a microfluidic device with a flow-focusing geometry. The continuous phase is silicon oil containing a surfactant, while the dispersed phase is an aqueous mixture of microgels and glutaraldehyde. (b) Brightfield image of partially dried emulsion droplets. The surface of the droplets gets crumpled as water evaporates from within the droplets, indicating the formation of a membranous shell at the oil–water interface. (c) A confocal microscope image of the aqueous droplets in silicon oil reveals distinct assembly of fluorescently tagged microgel particles along the oil–water interface. Fluorescence within the drops suggests the presence of excess microgels in the bulk of the droplets. A confocal microscope image of emulsion droplets containing one-tenth of the original concentration of microgels is included as an inset for comparison. (d) Fluorescence intensity profile across the cross section of droplets with two different microgel concentrations. Scale bars represent a length of 100 μm .

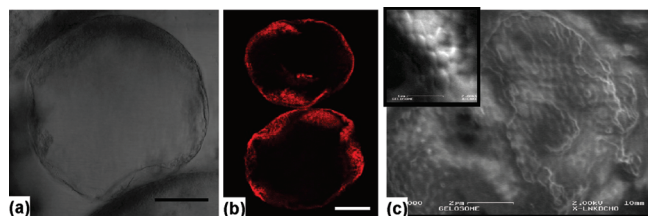


Figure 3. (a) Brightfield image of a PNIPAm colloidosome suspended in water. (b) Confocal microscope image of colloidosomes in water confirms their core–shell architecture. PNIPAm microgels are tagged with Rhodamine B for ease of visualization. (c) A scanning electron microscope (SEM) image reveals random packing of microgels along the shell of the colloidosomes. A high magnification SEM image is included as an inset for clarity. Scale bars represent a length of 25 μm in (a) and (b), 2 μm in (c), and 1 μm in the inset image.

being imaged with a digital camera attached to the microscope. Size data of three different colloidosomes are averaged for better statistics. For comparison, equilibrium size-change of the constituent microgels over the same temperature range is also measured using dynamic light scattering. The colloidosomes exhibit a thermosensitive behavior similar to that of their constituent microgels. On increasing the temperature from 20 to 55 $^{\circ}\text{C}$, the diameter of the colloidosomes decreases by 42% which roughly translates to a 80.5% decrease in volume (Figure 4). Upon cooling down to 20 $^{\circ}\text{C}$, colloidosomes regain their original volume with no significant hysteresis effects. The $\sim 80\%$ reduction in volume exhibited by colloidosomes upon actuation makes them attractive candidates for applications that require on-demand bulk release of materials in a short time. A movie

demonstrating the shrinking of a colloidosome upon increasing the temperature is included in the Supporting Information (Movie S2). Notably, the change in the size of the colloidosomes is not as great as that of the constituent microgels, which exhibit $\sim 49\%$ decrease in diameter. Also, the size change in colloidosomes occurs over a wider range of temperature as compared to that of the constituent microgels. We believe that covalent linking of the microgel particles by glutaraldehyde is responsible for these discrepancies. The chemical bonding between the microgel particles increases the effective cross-linking of the system which could affect its phase transition behavior.

To summarize, we have presented a novel technique to fabricate monodisperse stimuli-responsive colloidosomes using stimuli-sensitive microgel particles as building blocks. Key to this technique is the self-assembly of the microgel particles along the oil–water interface. The colloidosomes exhibit a reversible size-change behavior similar to that of their constituent microgels. Hence, they are attractive candidates for applications that require targeted pulsed-release of materials in cosmetics and pharmaceutical formulations. The technique is highly versatile, as it can be used in conjunction with microfluidics to produce highly monodisperse colloidosomes or with any other bulk-emulsification techniques to produce colloidosomes in large quantities; the polydispersity of the colloidosomes will vary depending upon the emulsification technique employed. Although we demonstrate fabrication of thermosensitive colloidosomes, the technique can be extended to fabricate colloidosomes that are responsive to other stimuli, such as pH, ionic strength, or electromagnetic fields, by incorporating appropriate functional groups inside or on the surface of the colloidal particles.

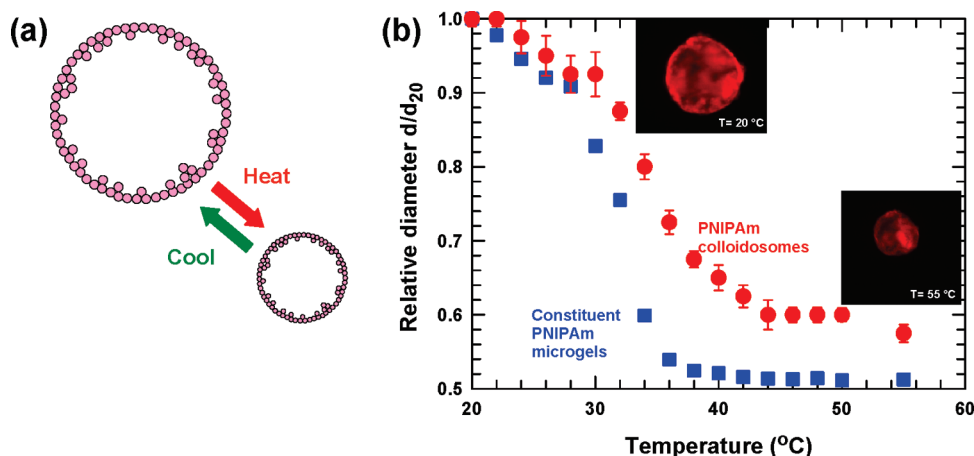


Figure 4. (a) Schematic representation of the thermoresponsive behavior of a PNIPAm colloidosome. (b) Equilibrium size change of PNIPAm colloidosomes and the constituent PNIPAm microgels. Colloidosomes were dispersed in water and heated from 20 to 50 $^{\circ}\text{C}$ in fixed increments of 2 $^{\circ}\text{C}$ and then to 55 $^{\circ}\text{C}$. Images were captured after allowing the sample to equilibrate for 30 min at each temperature. The sample was then cooled down to 20 $^{\circ}\text{C}$ using the same temperature steps. Dimensions of the colloidosomes were measured from the captured images using image-processing software. Size data of three different colloidosomes were averaged for better statistics. Size-change data of the constituent PNIPAm microgels over the same temperature range were collected using dynamic light scattering.

Experimental Section

Microgel Synthesis. PNIPAm microgels were synthesized by precipitation polymerization in a three-neck round-bottom flask. NIPAm monomer (4 g, 99% purity, Acros) was dissolved along with *N,N'*-methylene bisacrylamide (0.16 g, 99.5% purity, Fluka) and allyl amine (0.28 mL, Sigma-Aldrich) in deionized water (150 mL). The solution was filtered with a 1 μm filter and transferred to a round-bottom flask (250 mL) fitted with a stirrer, a condenser, and a nitrogen purge line. The solution was heated to 68 $^{\circ}\text{C}$ and allowed to equilibrate for 30 min under nitrogen. A fluorescent dye, methacryloxy thiocarbonyl rhodamine B (0.0004 g), dissolved in dimethyl sulfoxide (0.1 mL) and diluted with water (4.9 mL), was then added to the stirred mixture. This was followed by the addition of a reaction initiator, $\text{K}_2\text{S}_2\text{O}_8$ (0.2 g, 99% purity, Sigma-Aldrich) dissolved in water (5 mL). The monomers were allowed to polymerize for ~ 120 min. The nascent microgel suspension was filtered using a filter cloth (100 μm mesh size) and quickly cooled down to room temperature using an ice bath. The suspension was dialyzed for a week to remove unreacted monomers and dyes. The calculated PNIPAm microgel concentration in the aqueous suspension, based upon reactant concentration and assuming 100% conversion, is ~ 2.5 wt %; however, the actual concentration is estimated to be less (~ 1.4 wt %).¹⁴ Microgel particle size was determined using dynamic light scattering (Malvern Zetasizer Nano-ZS, 633 nm laser, 173 $^{\circ}$ scattering angle) and fluorescence optical microscopy (Leica TCS-SP5 scanning confocal microscope). A fluorescent micrograph of the synthesized microgels is included in the Supporting Information (Figure S1). Electrophoretic mobility of the microgels was determined also using the Malvern Zetasizer instrument.

Microfluidics. Detailed description of fabrication and operation of glass capillary based microfluidic devices for generating emulsions can be retrieved from prior publications.^{17,18,21} The aqueous phase for microfluidic emulsification was prepared by dissolving 1 vol % glutaraldehyde (50% w/v, Aldrich) in the microgel suspension. The continuous phase was poly(dimethylsiloxane) fluid, DC550 (125 cSt, 1.07 g/cm³, Dow Corning), containing 0.4 wt % DC547 for emulsion stabilization. Fluids were pumped into the microfluidic device using syringe pumps (Harvard PHD 2000 series). Flow rates for the continuous and the dispersed fluids were set at 2000 and 500 $\mu\text{L}/\text{h}$, respectively.

The microfluidics process was monitored using an inverted optical microscope (DM-IRB, Leica) fitted with a fast camera (Phantom V9, Vision Research). Although microgels assemble at the oil–water interface to stabilize the emulsion, the process is not rapid enough to prevent coalescence of droplets in the microfluidic device. Hence, addition of a small amount of surfactant (DC547) was necessary. The emulsion was heated in an oven at 60 $^{\circ}\text{C}$ for 30 min and then stored under ambient conditions for 72 h. Colloidosomes were removed from the silicon oil and repeatedly flushed with isopropanol to remove any adsorbed oil. To redisperse the colloidosomes in water, it was necessary to wash them with water. However, while washing with water, a large number of colloidosomes stick irreversibly to the sample vial (glass). PNIPAm microgels are known to adhere to glass surfaces possibly due to a combination of van der Waals interactions and hydrogen bonding between the hydroxyl groups on the glass surface and the amide groups on the particles.²² To alleviate this problem, we coated the inside of the glass vial with a hydrophobic layer of octadecyltrimethoxysilane. We also introduced an intermediate step wherein isopropanol was stripped off the colloidosomes with 1,4-dioxane. Since 1,4-dioxane is miscible with both water and isopropanol, it was possible to wash it off the colloidosomes with multiple washes with water. Although these steps improved the yield of colloidosomes, it did not completely suppress adhesion of colloidosomes to the glass surface during aqueous washes.

Characterization. Brightfield images of drying emulsion droplets were taken using an inverted optical microscope (DM-IRB, Leica) fitted with a camera (QImaging QICAM). Confocal images of the droplets and colloidosomes were obtained using a scanning confocal microscope (Leica TCS-SP5). Fluorescence intensity across the cross section of the droplets was determined using an image-processing software, ImageJ. SEM images were collected with a Zeiss Ultra55 field emission microscope (FESEM). Thermosensitive behavior of the colloidosomes was examined using a microscope mountable thermal stage (Physitemp Instruments, TS-4ER). For measuring equilibrium size change of colloidosomes, the colloidosomes along with Millipore water were placed in a transparent holder, which was placed on a thermal stage. The stage was mounted on an inverted microscope (DM-IRB, Leica) fitted with a camera (QImaging QICAM), and images were taken at regular temperature intervals of 2 $^{\circ}\text{C}$. Samples were

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allowed to equilibrate for 30 min at each temperature before imaging. Size data of three different colloidosomes were averaged for better statistics. Size-change data of the constituent microgels at different temperatures were collected using dynamic light scattering (Malvern Zetasizer Nano-ZS, 633 nm laser, 173° scattering angle).

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Supporting Information Available: Florescence micrograph of microgels synthesized by precipitation polymerization (Figure S1). Movie displaying a three-dimensional view of colloidosomes (MovieS1.avi). The movie was created by stacking a series of planar confocal images taken along the *z*-direction. Movie displaying the thermoresponsive behavior of PNIPAm colloidosomes (MovieS2.avi). This material is available free of charge via the Internet at <http://pubs.acs.org>.