# *In-Situ* Synthesis of Temperature-Sensitive Hollow Microspheres via Interfacial Polymerizations

*Yulin Deng,[1,2] Qunhui Sun[2]*

[1]*School of Chemical & Biomolecular Engineering,*

[2]*IPST at GT,*

*Georgia Institute of Technology, Atlanta, GA 30332-0620*

*Email: Yulin.Deng@chbe.gatech.edu, Qunhui.Sun.@ipst.gatech.edu*

## Introduction

In recent years, hollow microspheres have attracted intense research attention because of its wide variety of applications such as in delivery vesicles for drugs, dyes, or inks, microcontainers for artificial cells, and protection shield for proteins, enzymes, or DNA, and for catalysis applications as well.[1-3] Both inorganic and polymeric hollow microspheres have been reported. Templating method is one of the most common methods for preparing hollow spheres, but the application of this approach is limited because in most cases the materials that need to be encapsulated in the microspheres are not suitable templates.[4] Hubert and coworkers reported an approach with hydrophobic monomer entrapped between the liposome bilayer and followed by polymerization *in situ*, and Landfester, et al. reported a miniemulsion process followed by immediately hydrophobe removal.[4d,5] The shortcoming of these two methods is that only limited bilayer systems or hydrophobe-polymer pairs can be found. To our best knowledge, there have been no reports on one-pot polymerization to prepare temperature sensitive hollow microspheres via an interfacial polymerization approach under mild reaction conditions.

Poly(N-isopropylacrylamide) (PNIPAM) and its derivatives have attracted increasing research interests due to their intriguing temperature-sensitive performance.[6-9] Among the wide applications of the PNIPAMs, microspheres are of particular interests in terms of the state-of-the-art applications in controlled drug release,[7] biosensors and actuators,[8] and bioengineering material applications[9] as well. Nevertheless, most part of the researches are focused on the physical and chemical performances at around its lower critical solution temperature (LCST), or called coil-to-globule transition.[6] To date, little work was done using this transition in polymer reaction engineering to develop novel material architectures and functions. In this study, the
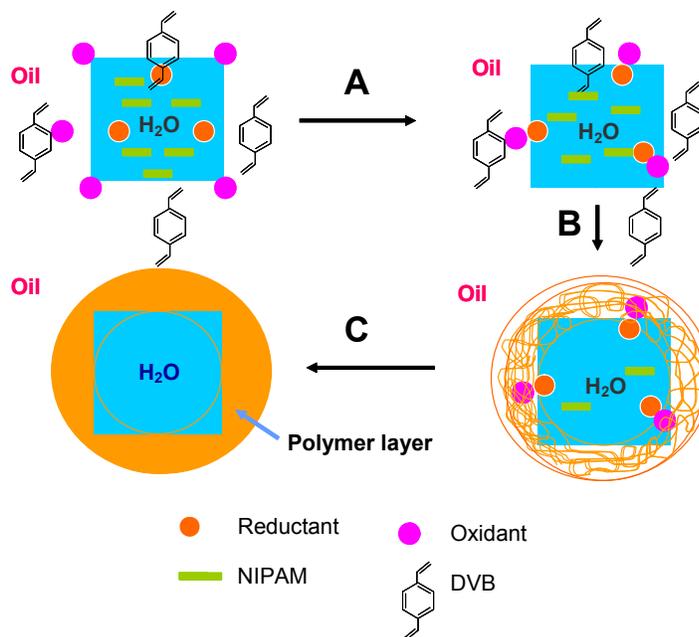


**Figure 1**. Schematic illustration of in-situ synthesis of temperature-sensitive hollow structured microspheres via an inverse emulsion polymerization approach.

transition of PNIPAM from hydrophilic to hydrophobic above its LCST was adopted in a designed unique interfacial polymerization process by which the temperature sensitive hollow microspheres could be synthesized *in situ*.

As depicted in Scheme 1, the NIPAM monomer was dissolved in an aqueous phase first and then emulsified with toluene to form a water-in-oil (W/O) emulsion in the presence of low HLB surfactant such as sorbitan monooleate (commercial name Span-80, with HLB value of 4.3). The emulsion droplets with diameter of 1-3 $\mu$m could be obtained. In order to conduct an interfacial polymerization at the oil/water interface, a redox initiation system containing benzoyl peroxide (BPO) in oil phase and tetraethylenepentamine (TEPA) in water phases was used as the interfacial initiator.[10] During the reaction process A as described in Scheme 1, the reductant TEPA and the oxidant BPO will diffuse to the oil-water interface first to generate free radicals. Following after that, the polymerization of NIPAM will start spontaneously at the interface. If the polymerization is carried out at the temperature above the LCST of PNIPAM, the PNIPAM will be neither water-soluble (hydrophobic) nor oil-soluble (toluene as oil phase) as shown in process B in Scheme 1. For this reason, the PNIPAM layer formed will be restricted at the oil-water interfacial area at the temperature above its LCST. At the same time, the crosslinking agent (divinylbenzene, DVB) in oil phase will also diffuse to the interface and participate in the polymerization. As the polymerization continues, all monomers and crosslinkers are reacted and an insoluble crosslinked PNIPAM network is formed at the interface with a hollow microspheric structure, as shown in Process C of Scheme 1. The suspension polymerization approach follows the same reaction and shell formation mechanism except that the oil phase is suspended in the water phase and a suspension agent is used.

## Experimental Part

**Materials:** Sorbitan monooleate (Span-80), divinylbenzene (DVB, technical grade, 80%, mixture of isomers), benzoyl peroxide (BPO, 97%), tetraethylenepentamine (TEPA, technical grade), Mowiol 40-88 (poly(vinyl alcohol), 88% hydrolyzed, $M_w \sim 127,000$), and toluene (99.8%) were purchased from Sigma-Aldrich, and used without further purification. The *N*-isopropylacrylamide (NIPAM, 97%) was from Sigma-Aldrich and recrystallized from benzene and hexane twice and used after vacuum drying.[11] De-ionized (DI) water (milli-Q grade) was used throughout the experiments.

**Preparation of Hollow Microspheres:** In a typical W/O run, 4.0 g of Span-80, 0.2 g of DVB, and 0.2 g of BPO were dissolved in 50 ml of toluene as the oil phase, and 1.0 g of NIPAM and 0.1 g of TEPA were dissolved in 5.0 ml of DI water as the water phase. The two individual phases were mechanically mixed in a blender (Hamilton Beach brand, model 52200, from Proctor-Silex, Inc.) at Liquefy grade for 5 min. The mixture was then poured into a 250 ml three-neck round-bottom flask equipped with a mechanical stirrer and a condenser. Under mechanical agitation (500 ± 10 rpm), the reaction was carried out at 60 °C for more than 4 hrs under nitrogen atmosphere. After that, the oil phase was separated under centrifugation (Beckman 20) at 8,000 rpm for 10 min, and the water phase was collected for further characterization. In a typical suspension polymerization run, 3.0 g of oil solution consisted of 55.0 wt.% of M-solvent, 40.0 wt.% of toluene, 2.7 wt.% of DVB, and 2.3 wt.% of BPO, was poured into an aqueous solution comprised of 15 ml of 0.5 wt.% of Mowiol 40-88 (cooked at 90 °C for 1 h and then filtered), 1.5 g of NIPAm, and 0.3 g of TEPA under vigorous magnetic stirring. The mixture was then raised to 60 °C under helium atmosphere and the reaction was carried out at this

temperature for more than 4 h under continuous magnetic stirring. The mixture after the reaction was then separated under centrifugation (8,000 rpm) , and washed with toluene and water respectively.

**Cryo-Breaking of Hollow Microspheres:** A cryo-breaking technique to tailored breaking the microspheres under ultrasonification in a liquid nitrogen medium was developed, and detailed as follows. A mother liquor containing the microspheres in a glass vial was solidified first by immersing into a liquid nitrogen medium and then partially melted by naturally exposing to the air. At this point, the vial was placed into an ultrasonification chamber, and let the tip of the ultrasonicator touching at the solid/liquid interface. The process was lasted for ca. 60 s. After that, the sample was ready for subsequent characterization.
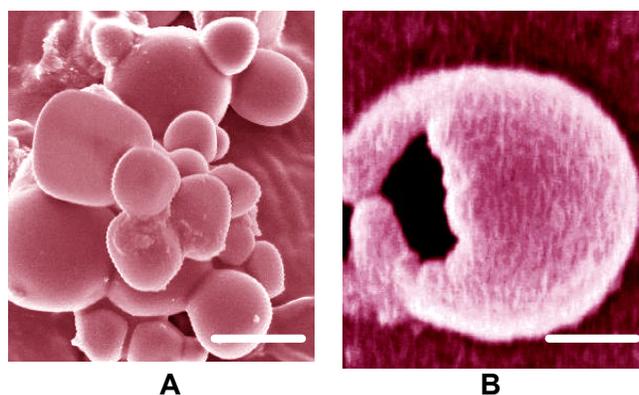


**Figure 1**. SEM images of PNIPAM microspheres. A: Overview picture; B: Cross section. Scale Bar: A: 2.0 $\mu$m; B: 1.0 $\mu$m.

**Instrumentation:** Scanning Electron Microscopy (SEM) was conducted on a LEO 1530 thermally-assisted field emission (TFE) scanning electron microscope SEM machine with an acceleration voltage of 3 kV. The samples were lightly sputter coated a thin layer of Au/Pd prior to the observation. Optical microscope observation was carried out on a Leica DMLM microscope equipped with a Leica DC 100 camera shot atop. Images taken with the camera shot was handled in a computer connected with a Leica IM 50 Image Manager. The samples were heated on the stage spontaneously with the optical observation and pictures taking. The ultrasonification was performed on a W-385 sonicator (Heat System-Ultrasonics, Inc., USA) with 1 s of cycle time, 70% of duty cycle, and an output of the microtip at level 5.
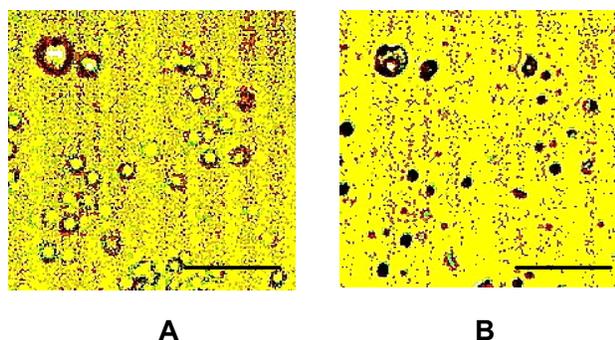
### Results and Discussion

As can be seen in Figure 1 A, the microspheres formed via the proposed approach have a quasi spherical morphology, with separated particles and microspheres diameters ca. 1-3 $\mu$m, in accordance with the particle size scale in an inverse emulsion process according to our previous studies.[12,13] The polydispersity of the particle size distribution of the microspheres, as shown in Figure 1 and other experiments (data not shown here) suggest that 1) the emulsion stability and drop size distribution are important for controlling the final product particle size
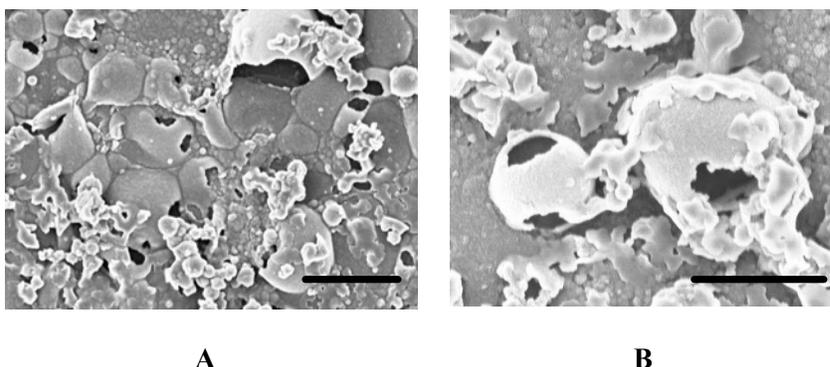


**Figure 2.** An overview (A) and close-up (B) vision of the hollow structured microspheres indicating a broken cross section profile and an empty interior structure. Scale bar: 3 $\mu$m.

distribution; 2) the agitation rate is another key factor because the formation of initial shell may be destroyed if too high share is used; and 3) The concentrations of crosslinker and monomer should not be relatively high, otherwise, the final product is not strong enough to be constructed as a hollowed sphere. Our preliminary experiments indicated that plethora broken and twisted microspheres were formed when insufficient or inappropriate surfactants were used. As expected, the interior morphology of the microspheres prepared according to the above mentioned procedures, as shown in



**A**            **B**

**Figure 3**. Optical microscope images of the microsphere particles at A: raised temperature (>LCST), and B: room temperature. Scale bar: 10.0 $\mu$m.

Figure 1B and Figure 2, did exhibit a truly hollow structure, with a wall thickness ca. 100 nm and particle size at ca. 1-3 $\mu$m, further supporting the feasibility of the concept proposed.

It has been well known that crosslinked PNIPAM is a temperature sensitive polymer network in water that will swell at the temperature lower than its LCST and deswell at the temperature higher than its LCST.[14,15] This is really the case when the hollow structured microspheres comprised mainly of PNIPAm prepared as described above. As expected, the size of the hollow structured microspheres changed dramatically upon temperature variation, approximately ca. 1 time around the LCST as demonstrated in Figure 2. The particles changed from transparent spots to dark one upon temperature increase and vice versa, indicating the reversibility and reproducibility of the swelling and deswelling behavior of the as-prepared hollow microspheres in the temperature changing course.[16,17]

Figure 4 illustrated an aerial (A)



**A**            **B**

**C**            **D**
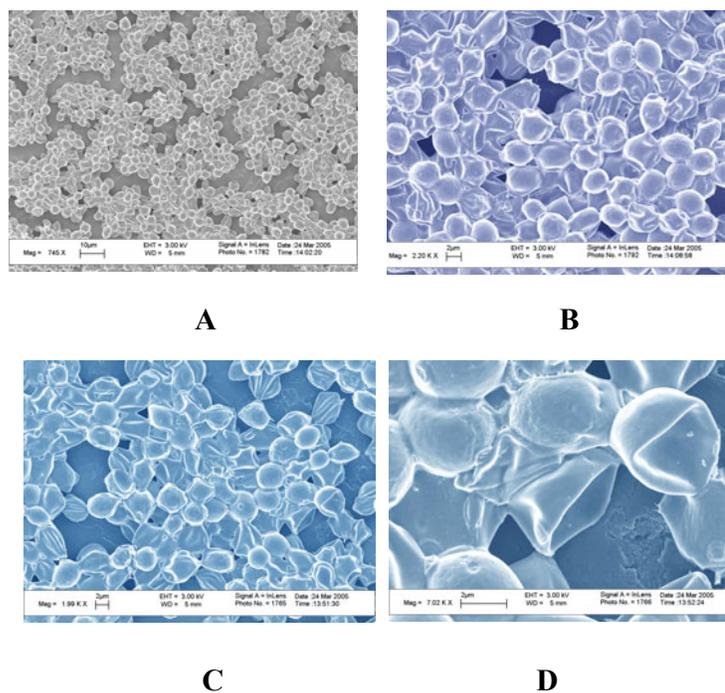
**Figure 4**. SEM pictures of temperature-sensitive hollowed structured microspheres prepared via suspension polymerization approach using Mowiol (40-88) as suspending agent.

and a zoom-in view (B) of the hollow structured microspheres prepared under suspension polymerization conditions. It was interesting to note that the microspheres had an average diameter of about 3 to 5 $\mu$m, about 2 $\mu$m larger than those prepared following an inverse W/O process. The appearance of the hollow microspheres, due to the strong evacuation under high vacuum condition for SEM observation, provided as flocks of individual flat balloons stacked together at random, further indication of the interior empty structure.

In conclusion, a novel one-pot synthetic strategy to prepare hollow structured PNIPAM microspheres via interfacial polymerization approaches at the interface of an inverse W/O emulsion or a suspension polymerization system has been demonstrated.  The results show that the PNIPAM microspheres prepared have a real empty core and a polymer shell structure, with a wall thickness of ca. 100 nm, and a size range of ca. 1-3 and 3-5 $\mu$m corresponding to W/O and suspension case respectively. The hollow structured microspheres experienced a reversible swelling and deswelling processes via mediating the temperature below and above the LCST.  The particle size and the thickness of wall depend on the emulsion stability, the network formation rate and the polymer concentration in the emulsion droplet.  Too high shear force will prevent the formation of polymer shell at the surface of the dispersed droplets.  Because of the flexibility in choosing substances being dissolved in the water phase, this approach revealed perspective in impregnating bioactive materials or drugs requiring mild encapsulation conditions within a temperature sensitive polymer shell.   The methodology demonstrated in the study not only provided a ubiquitous technical pathway in hollow microspheres construction with one-pot approach under mild reaction conditions, but also provided a new platform in understanding the diffusion and migration mechanism of PNIPAM at an oil/water interface above its LCST, and the polymer layer formation mechanism as well.  The ongoing works are being under way.

## References

(1)    (a) Tamber, H.; Johansen, P.; Merkle, H. P.; Gander, B. *Adv. Drug Delivery Rev.* **2005**, *57*, 357-376. (b) Trehan, A.; Sinha, V. R. *J. Control Release* **2003**, *90*, 261-280. (c) Tracy, M. A. *Biotechnol. Progr.* **1998**, *14*, 108-115.

(2)    (a) Raymond, M-C.; Neufeld, R. J.; Poncelet, D. *Artif. Cell Blood Sub.* **2004**, *32*, 275-291. (b) Hammer, D.A.; Discher, D.E. *Annu. Rev. Mater. Sci.* **2001**, *31*, 387-404.

(3)    (a) Molvinger, K.; Quignard, F.; Brunel, D.; Boissiere, M.; Devoisselle, J-M. *Chem. Mater.* **2004**, *16*, 3367-3372. (b) Zhang, J.; Xu, S.; Kumacheva, E. *J. Am. Chem. Soc.* **2004**, *126*, 7908-7914.

(4)    (a) Mueller, A.; O'Brien, D. F. *Chem. Rev.* **2002**, *102*, 727-757. (b) Shenoy, D. B.; Antipov, A. A.; Sukhorukov, G. B.; Möhwald, H. *Biomacromolecules* **2003**, *4*, 265-272. (c) Johnson, S. A.; Ollivier, P. J.; Mallouk, T. E.. *Science* **1999**, *283*, 963-965. (d) Hubert, D. H. W.; Jung, M.; German, A. L. *Adv. Mater.* **2000**, *12*, 1291-1294. (e) Bronich, T. K. ; Ouyang, M. ; Kabanov, V. A. ; Eisenberg, A. ; Szoka Jr., F. C.; Kabanov, A. V. *J. Am. Chem. Soc.* **2002**, *124*, 11872-11873.

(5)    Tiarks, F.; Landfester, K.; Antonietti, M. *Langmuir* **2001**, *17*, 908 -918.

(6)    (a) Pelton, R. H. ; Chibante, P. *Colloids Surf.* **1986**, *20*, 247. (b) Zhang, J.; Pelton, R.; Deng, Y. *Langmuir*, **1995**, *11*, 2301-2302.

(7)    Huang, G.; Gao, J.; Hu, Z.; St. John, J. V.; Ponder, B. C.; Moro, D. *J. Control Release* **2004**, *94*, 303-311.

(8)    Cheng, X.; Wang, Y.; Hanein, Y.; Bohringer, K. F.; Ratner, B. D. *J. Biomed. Mater. Res. - A* **2004**, *70*, 159-168.

(9)    Ohya, S.; Sonoda, H.; Nakayama, Y.; Matsuda, T. *Biomaterials* **2005**, *26*, 655-659.

(10)   Luo, Y.; Schork, J. F; Deng, Y.; Yan, Z. *Polym. Reaction Eng.* **2001**, *9*, 183-197.

(11)   Hu, T.; You, Y.; Pan, C.; Wu, C.*; J. Phys. Chem. B*. **2002**, *106*, 6659-6662.

(12)   Sun, Q.; Deng, Y. *J. Colloid Interface Sci.* **2004**, *278*, 376-382.

(13)   Hu, Zeshan; Deng, Yulin; Sun, Qunhui *Colloid J.*  **2004**, *66*, 745-750.

(14)   Hoare, T.; Pelton, R. *Macromolecules* **2004**, *37*, 2544-2550.

(15)   Jones, C. D.; Lyon, L. A. *Macromolecules* **2003**, *36*, 1988-1993.

(16)   Stieger, M.; Richtering, W. *Macromolecules* **2003**, *36*, 8811-8818.

(17)   Zhang, W.; Zhou, X.; Li, H.; Fang, Y.; Zhang, G. *Macromolecules* **2005**, *38*, 909-914.