Synthesis of Thermoresponsive Copolymers Composed of Poly(ethylene glycol) and Poly(N-isopropyl acrylamide) for Cell Encapsulation

Tatiya Trongsatitkul and Bridgette M. Budhlall

NSF NSEC Center for High-Rate Nanomanufacturing, Department of Plastics Engineering, University of Massachusetts-Lowell, Lowell, MA 01854, U.S.A.

ABSTRACT

Thermoresponsive copolymers of poly(*N*- isopropyl acrylamide) (PNIPAm) and poly(acrylamide) microgels copolymerized with poly(ethylene glycol)(PEG) chains were synthesized by free-radical photopolymerization. Poly(ethylene glycol) methyl ether methacrylate (PEGMA) macromonomers with varying number-average molecular weights were used (M_n = 300 and 1,000 g/mol). A simple microarray technique coupled with a laser scanning confocal microscope (LSCM) was used to visualize the effect of temperature on the volume phase transition temperatures of the microgels. In general, increasing the concentration of PEGMA in the PNIPAm-*co*-Am-*co*-PEGMA copolymers resulted in a broader and higher volume phase transition temperature (PVTT) compared to the PNIPAm microgels. We demonstrated that the PEGMA molecular weight and concentration influenced whether it was incorporated as a grafted copolymer or random copolymer in the PNIPAm microgel. The evidence for this is the shift in PVTT as determined by temperature response and differential scanning calorimetry (DSC) measurements. This behavior suggests that incorporation of PEGMA in the copolymer depends on its hydrophilicity or water-solubility which in turn influenced the degree at which the copolymer chains collapsed from a coil-to-globule (volume phase transition) with increasing temperature.

INTRODUCTION

Poly(*N*- isopropyl acrylamide) (PNIPAm) is a well known temperature responsive polymer. PNIPAm demonstrates a volume phase transition temperature (VPTT) or lower critical solution temperature (LCST) in aqueous solution at about 32°C [1]. At temperatures above LCST, the PNIPAm chains collapse due to dissociation of water as PNIPAm becomes more hydrophobic. PNIPAm microgels with a characteristic LCST close to biological temperatures is attractive for various biomedical applications including controlled drug delivery release, tissue regeneration and cell encapsulation.

Cell encapsulation is one of the most promising potential biomedical applications of PNIPAm microgels. In cell encapsulation, transported cells are protected from immune rejection by an artificial, semi-permeable membrane, potentially allowing transportation without the need for immuno-suppression [2]. To improve the biocompatibility of PNIPAm microgels, surface modification is needed to render stealth properties to the microgel by coating or grafting with biocompatible polymers, such as poly(ethylene glycol)(PEG) or poly(ethylene oxide)(PEO). The stealth character of PEG is based on its steric repulsion properties. In aqueous solution, the oxygen on PEG segments form hydrogen bonds with water, providing the PEG segments with a protective hydration shell that minimizes the non specific interactions of the modified microgels within the biological environment [2, 3].

Incorporation of PEG with PNIPAm microgels achieves synergist benefits of biocompatibility from PEG and thermal responsive characteristics from PNIPAm, respectively.

However, PEG incorporation may alter the LCST or VPTT of PNIPAm. LCST is principally dependent on the hydrogen bonding capability of the constituent monomer units. Thus, incorporating PEG can be expected to change the hydrophilic/hydrophobic characteristic of PNIPAm leading to a shift in the LCST or VPTT with increasing temperature.

The goal of this research is to understand the effect of grafting PEG macromonomer to PNIPAm on the volume phase transition temperature of the grafted copolymer microgel. These PNIPAm-*co*-PEGMA copolymers will then be explored as thermoresponsive microgels for encapsulating mammalian cells for controlled delivery and release applications.

EXPERIMENT

Materials

All of the chemicals used in these experiments were obtained from Sigma Aldrich and used as received, unless otherwise noted. *N*-isopropylacrylamide (NIPAm), acrylamide (Am), poly(ethylene glycol) methyl ether methacrylate (PEGMA) (Mn = 300 and 1,000), *N,N'*-methylenebisacrylamide (BIS), 2,2-dimethoxy-2-phenylacetophenone (oil-soluble photoinitiator), anthraquinone-2-sulfonic acid, sodium salt monohydrate (water-soluble photoinitiator), fluorescein isothiocyanate (FITC) dye, mineral oil, deionized water (Millipore Elix® 3).

Synthesis of PNIPAm-*co*-PEG

In this research, the effect of incorporating PEG macromonomer with PNIPAm-*co*-Am on the volume phase transition temperature (PVTT) of the microgel was investigated. The number average molecular weight (M_n) and concentration of PEGMA were varied. *N*-isopropylacrylamide (NIPAm) was copolymerized with acrylamide (AM) in a 95:1 molar ratio to increase the LCST of the resulting PNIPAm-*co*-Am copolymer temperatures closer to body temperature [4]. The effect of varying the molecular weight of PEGMA macromonomer at three different concentrations (10, 20, and 30 wt%) was also investigated. A crosslinker, *N,N'*-methylenebisacrylamide (BIS) in a molar ratio of 1:750 (crosslinker: monomers) was used to increase the molecular weight of the copolymer in addition to render the copolymer water-insoluble Oil-soluble and water-soluble photoinitiators (1:1 molar ratio) at 3000 ppm (based on monomer) were used to initiate the polymerization reaction. FITC dye was used to label the monomer droplet under epifluorescence mode of LSCM.

A simple microarray technique was used that enabled real-time observation of the polymerization and the thermal response of microgel under the optical microscope. As such, only a small quantity of chemicals is required for an experiment using this method. This is a major advantage to using this technique for exploratory research. A schematic of the microarray technique used to synthesize the PEG –*g*-PNIPAm microgels is shown in Figure 1. First, mineral oil was placed in a concave well on a poly(dimethyl siloxane) (PDMS) coated microslide. Second, a small drop (0.8 µl/drop) of monomer solution, prepared as described above, was placed onto the mineral oil. The monomer solution drops initially sink into the oil and serve as self-contained micro-reactors. Photopolymerization was conducted using UV irradiation. Observations under LSCM were then made every 30 mins. The microgels were completely polymerized after 90 mins of UV irradiation.

The temperature response of the microgels was then characterized by observations under the LSCM. A hot-stage (Instec WS60) was used to vary and control the temperature of the

microslides containing the microgels. The temperature was varied from 25 to 55°C at 5°C increments. The microgel samples were allowed to reach temperature equilibrium for 10 mins before microscope images were acquired. These images were then analyzed to determine the microgel diameters for comparison.

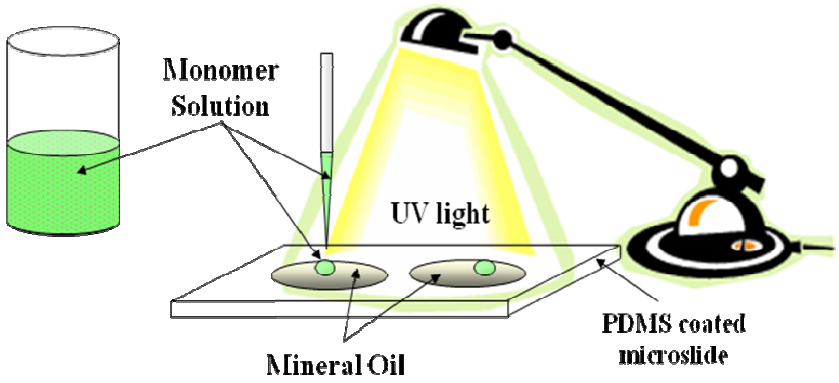


Figure 1. Schematic of microarray technique used for real-time observation of the polymerization

DISCUSSION

The VPTT of PNIPAm can be varied by copolymerization. In the present study, acrylamide was incorporated to achieve a PNIPAM-*co*-Am microgel with VPTT in the range of 38-41°C which is slightly above physiological body temperature of 37°C [5]. By incorporating another hydrophilic monomer-PEGMA, it is expected that the VPTT will be further increased. The extent of increase in VPTT can be adjusted by varying the concentration of the hydrophilic monomer. A summary of the experiments performed with PNIPAm-*co*-Am microgels synthesized using PEGMA with two different molecular weights and various concentrations is shown in Table I.

Table I. Experimental Parameters and Observations for PNIPAm-*co*-Am Microgels Copolymerized with PEGMA

Property	Control PNIPAm-*co*-Am	Low molecular weight-PEGMA (M_n = 300 g/mol)			High molecular weight-PEGMA (M_n = 1,000 g/mol)		
		10 wt%	20 wt%	30 wt%	10 wt%	20 wt%	30 wt%
VPTT	~35 °C	~40°C	~45°C	~50°C	~40°C	~35°C	~32°C

The effect of PEGMA number average molecular weight (M_n) on the VPTT of the grafted PNIPAm microgels was studied. A series of microgels were prepared without PEGMA and with 300 and 1,000 g/mol PEGMA respectively. They were all dispersed in a continuous phase of mineral oil as illustrated in Figure 1. The effect of PEGMA concentration on the volume phase transition of the PEGMA-*co*-Am microgel after exposure to temperatures ranging from 25-55°C was also studied. The behavior of these microgels suspended in mineral oil is illustrated for only (3) temperatures in Figure 2.

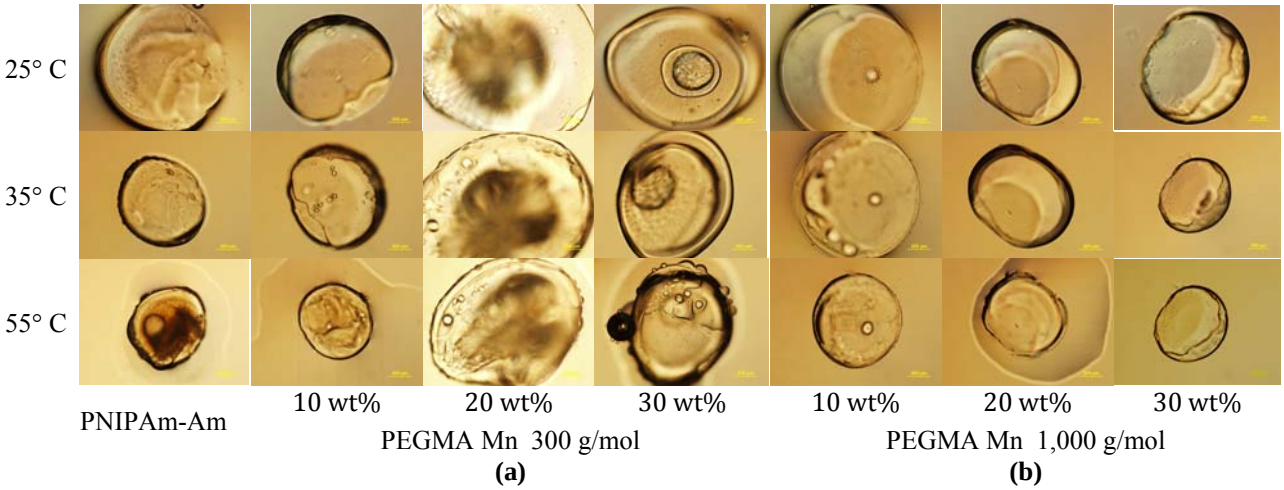


Figure 2. Optical micrographs of PNIPAm-*co*-Am microgels prepared with increasing concentration of (a) low M_n PEGMA (300 g/mol) and (b) high M_n PEGMA (1,000 g/mol).

In general, it was observed that the dimensions of all the microgels decreased with increasing temperature. The normalized difference in diameter is plotted as a function of temperature for the entire temperature range measured (25-55°C) for PNIPAm microgels copolymerized without and with PEGMA macromonomers with low M_n (Figure 3) and high M_n (Figure 4), respectively.

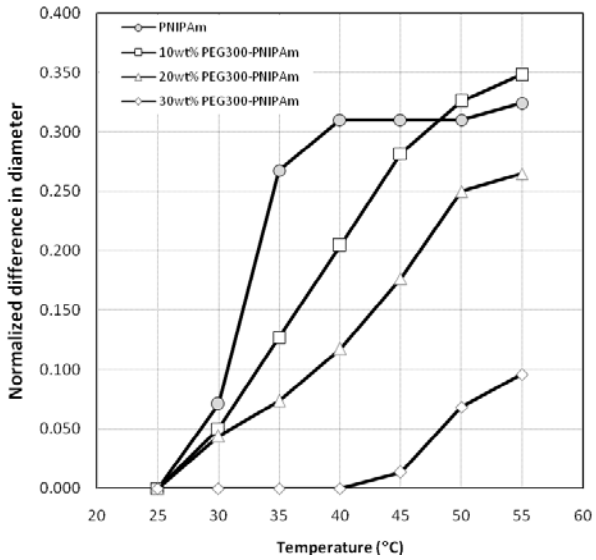


Figure 3. The comparison of temperature-induced shrinkage of PNIPAm microgels with 0 wt% (○), 10 wt% (□), 20 wt% (Δ), and 30 wt% (◊) PEGMA (M_n = 300g/mol).

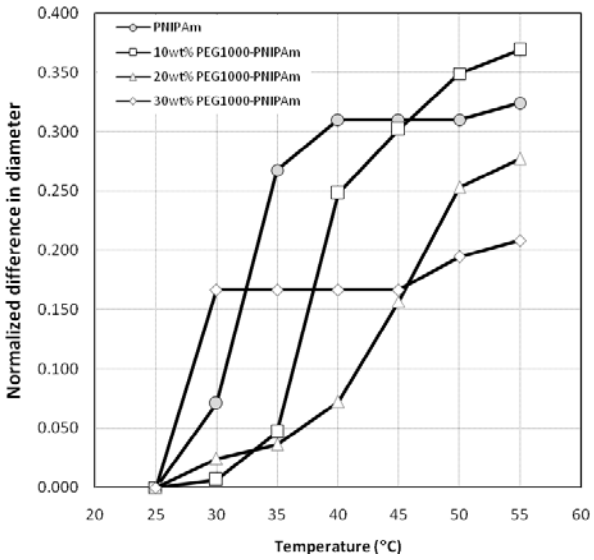
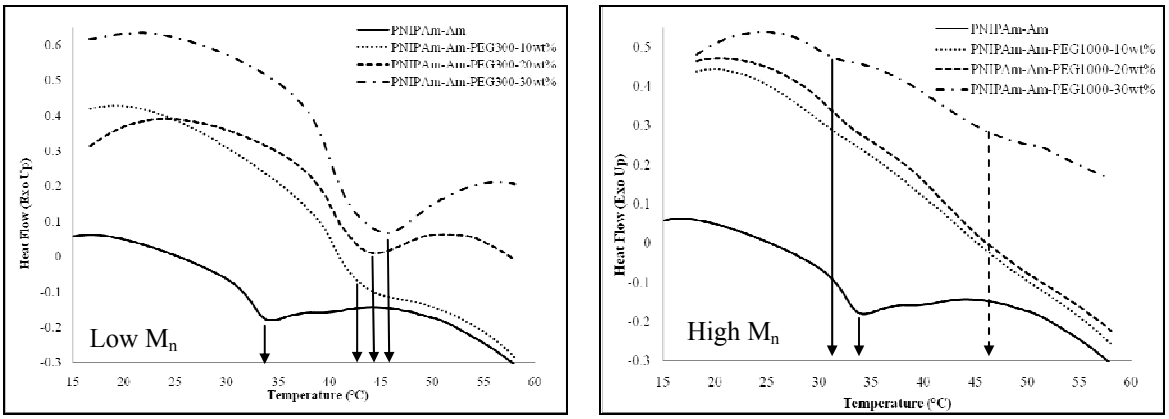


Figure 4. Comparison of temperature-induced shrinkage of PNIPAm microgels with 0 wt% (○), 10 wt% (□), 20 wt% (Δ), and 30 wt% (◊) PEGMA (M_n=1,000g/mol).

The VPTT of the microgels prepared with low M_n PEGMA increased and broadened with increasing concentration. In contrast, the VPTT of the microgels prepared with high M_n PEGMA, decreased and sharpened with increasing concentration in the range of 10-30 wt%. This behavior can be explained by the decrease in tendency for the hydrophobic associations of isopropyl groups of PNIPAm segments when hydrophilic comonomer units are incorporated. This in turn led to a broader VPTT that occurred at a higher temperature [3]. Similar trends were also found when other hydrophobic comonomers were incorporated into PNIPAm [1, 3, 6, 7]. When the highest concentration (30 wt%) PEGMA macromonomer at the highest molecular weight (Mn = 1,000 g/mol) was used; the VPTT persisted at 32 °C, a temperature closer to the characteristic LCST of pure PNIPAm. A plausible hypothesis for these results is that the M_n influences the miscibility of the two comonomers. As the M_n of PEGMA increased from 300 g/mol to 1,000 g/mol, the PEGMA became more hydrophobic and therefore less soluble. At higher concentrations this immiscibility enhanced the phase separation of PNIPAm. Therefore, the VPTT shown is closest to the LCST of pure PNIPAm due to its greater insolubility at the highest concentration and phase separation. This hypothesis is further confirmed by DSC as shown in Figures 5 (a) and (b).

The DSC thermograph of PNIPAm-*co*-Am (Figure 5 (a)) reveals an endothermic peak at 33.7 °C indicating the VPTT of PNIPAm-*co*-Am. As expected, incorporating PEGMA (M_n=300 g/mol) resulted in increasing of VPTT to ~45 °C with a broader transition. This result is in agreement with the observations made using LSCM. The single peak found in the DSC themographs of PNIPAm-*co*-Am copolymerized with PEG (M_n=300 g/mol) suggests that the resulting product is "random" copolymer. On the other hand, thermographs of PNIPAm-*co*-Am copolymerized with PEGMA (M_n 1,000 g/mol) (Figure 5 (b)) shows two endothermic peaks, one at ~32 °C and the other at ~45 °C. These (2) peaks are well defined in the thermograph of PNIPAm-Am with the highest concentration of PEGMA (1,000 g/mol), 30 wt%. This indicates that the obtained copolymers using higher Mn PEGMA are a mix of "*random*" copolymer and "*block*" copolymer. The DSC thermographs confirm the effect of molecular weight on phase separation of the two comonomers on the copolymerization mechanism as hypothesized.



(a) **(b)**

Figure 5. DSC thermographs of PNIPAm-*co*-PEG micogels (a) PEG (M_n=300g/mol) and (b) PEG (M_n=1,000 g/mol)

CONCLUSIONS

PNIPAm-*co*-PEGMA microgels were synthesized using free radical photo-polymerization via microarray technique. Incorporation of PEGMA macromonomer was found to broaden and shift the volume phase transition temperature toward a higher temperature as compared to the characteristic LCST of PNIPAm. It is believed that the PEGMA increased the hydrophilicity of the PNIPAm-*g*-PEGMA microgels and hindered the association of the isopropyl groups of PNIPAm segments, resulting in a higher LCST for the low M_n PEGMA. The poor solubility of the comonomers is believed to play an important role in the phase transition temperature of the microgels as illustrated by the highest concentration of PEGMA macromonomer (M_n = 1,000 g/mol). These microgels exhibit a similar phase transition temperature of about 32°C, close to that of the control (pure) PNIPAm microgels.

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