# Development of a Thermosensitive Grafted Drug Delivery System—Synthesis and Characterization of NIPAAm-Based Grafts and Hydrogel Structure

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**ABSTRACT:** A thermosensitive grafted hydrogel was investigated for heating-activated drug release. The hydrogel was created by grafting oligomers of *N*-isopropylacrylamide-*co*-acrylamide (AAm) to a poly(2-hydroxyethyl methacrylate), or PHEMA, hydrogel. *N*-Isopropylacrylamide-*co*-AAm oligomers were synthesized with a range of compositions to raise the lower critical solution temperature (LCST) above physiological temperature. PHEMA hydrogels with these thermosensitive grafts were synthesized by free-radical solution polymerization, using an acrylated version of the oligomers. The oligomers were characterized for their molecular weight, LCSTs, and rate of response to a change in temperature. With the flexibility in tuning their properties by varying reaction parameters, these oligomers present possibilities in several fields, including drug delivery. The impact of cross-linking agent type and the amount and presence of grafts on the polymer network structure was found by determining the hydrogel mesh sizes. PHEMA gels cross-linked with methylenebisacrylamide had larger mesh sizes than those cross-linked with ethylene glycol dimethacrylate. Increasing amounts of cross-linking agent decreased mesh sizes. LCSTs exhibited by oligomers were slightly lower than those exhibited by polymer gels of the same composition. The grafting reaction was found to have only a slight impact on the hydrogel mesh size. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 120: 1597–1606, 2011

**Key words:** stimuli-sensitive polymers; thermoresponsive behavior; drug delivery systems; grafted copolymers

## INTRODUCTION

Polymers have several desirable properties such as their mesh structure, tissue-like nature, and their ability to be combined with targeting vectors or drugs. Owing to their many properties such as response to stimuli, biocompatibility, mechanical strength, hydrophilicity, and hydrophobicity, polymeric systems have been considered attractive materials for several applications such as drug delivery, cancer therapy, bioseparations, and tissue engineering applications.[1–4] Lower critical solution temperature (LCST) polymers respond to a change in temperature by undergoing a change in their physical structure by phase separating from water with an increase in temperature. LCST homopolymers based on *N*-isopropylacrylamide (NIPAAm) have an LCST of 32°C and have been investigated in the design of drug-delivery systems over the past few decades.[5–14]

Because of the abrupt and reversible nature of phase transition from hydrophilic to hydrophobic at its LCST, PNIPAAm has been used in developing drug-delivery devices that can release the entrapped drug at temperatures below its LCST when it is hydrated and expanded but not release at temperatures above its LCST when it is dehydrated and collapsed. PNIPAAm hydrogels exhibit what is termed negative thermal response, which means the hydrogel releases the drug in response to a decrease in temperature as opposed to an increase in temperature. The challenge with negatively thermosensitive systems is that the physiological temperature would have to be decreased to activate the system to start releasing the drug. Yoshida et al.[15] fabricated a positive thermosensitive pulsatile drug-delivery device by enclosing the drug-loaded poly(NIPAAm-*co*-acrylamide) gel within an impermeable capsule with a release orifice. At lower temperatures, drug release is low because the gel completely occupies the inner volume of the capsule plugging the release hole; thus, the drug has to diffuse through the gel phase to pass out of the capsule. At higher temperatures, the gel shrinks, creating a gap between the gel and capsule, thereby increasing the effective surface area for drug release. Gutowska et al.[16] designed a device based on a squeezing concept similar to the one described by Yoshida et al.[15]

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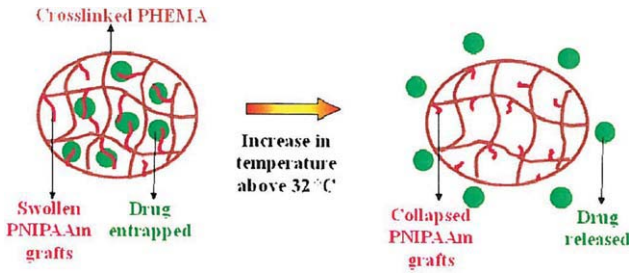


**Figure 1** Schematic representing the release of the drug from the grafted system with increase in temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

On–off release profiles of acetaminophen from a NIPAAm/acrylamide (AAm) gel show modulation in response to a temperature change between 35°C and 40°C. Release of acetaminophen was observed at 40°C (on phase) because of abrupt deswelling and mechanical squeezing of drug solution out of the matrix. When temperature was lowered to 30°C (off phase), release stopped because the release orifices were plugged by the swollen gel.

Much of the literature on PNIPAAm-based materials has been devoted to their potential uses in biomedical applications. For instance, it is fairly straightforward to modify the LCST by the addition of hydrophilic or hydrophobic comonomers.[9,11,17–19] However, even with an LCST above physiological conditions, thermally triggered release remains a challenge, particularly when it is desirable to have minimal release in an "off" state at 37°C. Positive thermosensitive designs, meaning that the drug is released as temperature rises, have focused on squeezing systems such as those described by Yoshida et al. and Gutowska et al. Although upper critical solution temperature polymers could fill this void, the existence of a polymer displaying a sharp phase separation in aqueous media has yet to be discovered. Recent work has focused on creating inert materials with thermoresponsive polymers to fill the pores in a membrane or as we propose here, grafts in the mesh space of a hydrogel. The grafted hydrogel works (in theory) is depicted in Figure 1. At low temperatures, the expanded grafts fill the mesh of the hydrogel, but when heated above the LCST, the grafts collapse, opening space for drug diffusion and release. Here, we investigate thermally responsive oligomers of NIPAAm-*co*-AAm and grafted hydrogels of P(HEMA-*g*-(NIPAAm-*co*-AAm)) that can be used in a positive thermoresponsive system where drug release is activated by heating, such as that which can be done by photothermal or magnetothermal therapy.

## EXPERIMENTAL

Oligomers with varying compositions of NIPAAm and AAm were synthesized by free-radical solution polymerization and characterized for their composition, molecular weight, LCST, and rate of response to temperature. P(HEMA-*g*-(NIPAAm-*co*-AAm)) hydrogels were synthesized with different cross-link agents and with a range of cross-link densities and were characterized for their nanostructure.

### Materials

NIPAAm, 2-hydroxyethyl methacrylate (HEMA), and AAm were obtained from Acros Organics (Fair Lawn, NJ). HEMA was purified using an inhibitor remover column to remove methoxy ether hydroquinone; all other chemicals were used as received. The redox initiators ammonium persulfate (AmPS) and sodium metabisulfite (NaMBS) and the thermal initiator benzoyl peroxide were obtained from Acros Organics. Methanol and toluene, which were used as solvents for polymerizations were obtained from Fisher Scientific (Fair Lawn, NJ). Methylenebisacrylamide (MBAAm) and ethylene glycol dimethacrylate (EGDMA), both cross-linking agents, and acryloyl chloride, which was used to activate the functional group on oligomers, were obtained from Aldrich Chemical Company, (Milwaukee, WI). 2-Aminoethanethiol hydrochloride, a chain transfer agent used to terminate polymerization of oligomers, was obtained from Acros Organics. Tetrahydrofuran (THF; Fisher Scientific) was used as a solvent (mobile phase) in gel permeation chromatography (GPC).

### Oligomer synthesis

Oligomers of NIPAAm and NIPAAm-*co*-AAm were synthesized by free-radical solution polymerization of NIPAAm and AAm in toluene. 5 wt % monomer was dissolved in 95 wt % solvent (toluene). 1 wt % thermal initiator benzoyl peroxide and 1 wt % chain transfer agent, aminoethanethiol hydrochloride, were added, and the mixture was stirred for 10 min to completely dissolve the reactants. The mixture was polymerized for 4 h at 90°C. Oligomer samples were recovered after decanting the solvent. Excess toluene was then added to dissolve the unreacted monomers. The oligomers settled to the bottom, whereas the solvent was decanted, and the product dried in a vacuum desiccator. Oligomers of NIPAAm-*co*-AAm were synthesized using 0, 9.2, 13.1, 16.7, and 20 mol % AAm.

### Oligomer functionalization

Oligomers obtained as described in the previous section were dissolved in 50 vol % aqueous methanol to which 1 wt % acryloyl chloride was added. The reaction mixture was placed in an ice water bath and continuously stirred while purging with nitrogen gas. The reaction was carried out at 4°C for

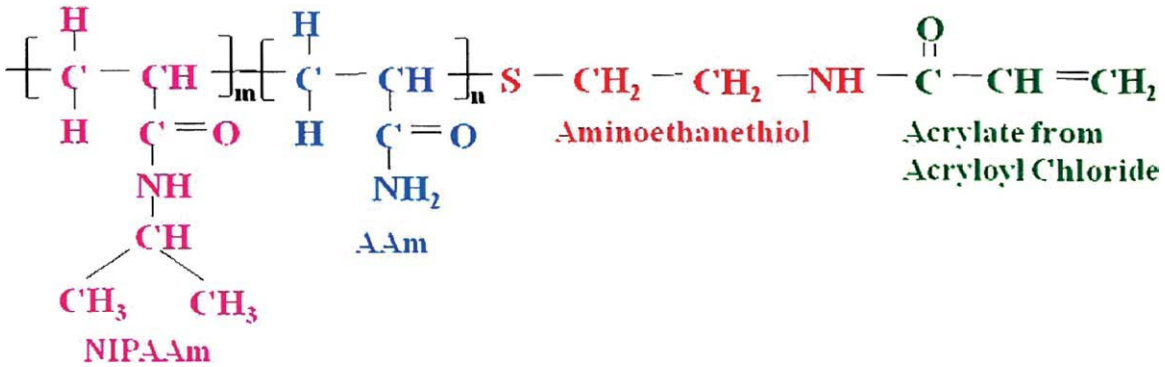


**Figure 2** Structure of (NIPAAm-*co*-AAm) oligomers terminated by aminoethane thiol and activated for free-radical polymerization using acryloyl chloride. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

approximately 1 h. Acrylation of the terminal amine enables the addition of a polymerizable double bond to the (NIPAAm-*co*-AAm) oligomer (Fig. 2). This double bond acts as a functional group enabling free-radical polymerization with HEMA monomer, allowing the oligomer to be grafted onto poly(2-hydroxyethyl methacrylate) (PHEMA) chains.

### Hydrogel synthesis

P(NIPAAm-*co*-AAm) hydrogel samples were prepared by free-radical polymerization in the presence of a cross-linking agent using feed AAm fractions of 0, 9.2, 13.1, 16.7, 20, 30, and 40 mol %. Monomers were dissolved in 50 wt % solvent (50 vol % methanol and 50 vol % water) to which 1 mol % cross-linking agent EGDMA was added. Sample solutions were purged with nitrogen gas with a syringe attached to a nitrogen gas-filled balloon to remove dissolved oxygen (a free radical scavenger) and 1 wt % (of monomer) redox initiators AmPS and NaMBS were added to it. The mixture was placed between siliconized glass plates with a 0.8-mm-thick Teflon® gasket and allowed to polymerize for 24 h at room temperature (25°C). PHEMA hydrogels were synthesized by dissolving 50 wt % HEMA monomer in 50 wt % solvent (50 vol % methanol and 50 vol % water) to which 1–15 mol % cross-linking agent (MBAAm or EGDMA) was added. The mixture was purged with nitrogen gas and 1 wt % redox initiators AmPS and NaMBS were added. The mixture was then placed between siliconized glass plates with a 0.8-mm-thick Teflon gasket and allowed to polymerize for 24 h at room temperature (25°C). Grafted hydrogels were synthesized in a similar manner to PHEMA gels. Here, 99 mol % HEMA monomer was dissolved in 1 mol % functionalized oligomers (with solvent added during the acrylation step, described below) and stirred continuously. 1 mol % EGDMA was added and nitrogen gas was purged to remove dissolved oxygen followed by addition of 1 wt % each of AmPS and NaMBS. The mixture was allowed to polymerize for 24 h at room temperature in between siliconized glass plates lined with a Teflon gasket. For each of the gel systems, the polymers were recovered after polymerization, stamped out into either disc-shaped (15 mm diameter by 0.8 mm thick) or dog-bone shaped samples and rinsed thoroughly in deionized water.

### Equilibrium swelling

PHEMA, P(NIPAAm-*co*-AAm), and P(HEMA-*g*-P(NIPAAm-*co*-AAm)) hydrogels of 15 mm diameter (measured in their relaxed state immediately after synthesis) were equilibrated for 24–48 h in deionized water at temperatures ranging between 15°C and 50°C, and their weights were measured at each temperature after blotting to remove surface water. The gels were then dried to constant weight in a vacuum desiccator, and their dry weights were measured. Weight swelling ratios ($q$) of the samples were calculated by dividing the wet weight at each temperature by the dry weight. The polymer volume fractions in the hydrogels' relaxed and equilibrium-swollen states ($\upsilon_{2,r}$ and $\upsilon_{2,s}$, respectively) was determined by weighing freshly synthesized (relaxed) or equilibrium-swollen samples on an analytical balance ($w_a$) and in a nonsolvent (hexane) using a density determination kit ($w_h$). From these two weights, and knowing the density of the nonsolvent, $\rho_h$, the volume was calculated according to Archimedes principle:

$$V = (w_a - w_h)/\rho_h \quad (1)$$

A similar procedure was used to determine the volume of dry hydrogel samples. Volume fractions were calculated by dividing the dry sample volume by the volume in either the relaxed or equilibrium-swollen state.

### Fourier transform infrared spectroscopy

The structure of the synthesized (NIPAAm-*co*-AAm) oligomers was characterized using Fourier transform infrared (FTIR) spectroscopy (Perkin-Elmer Spectrum™ 100 FTIR; Perkin-Elmer Life and Analytical Sciences, Shelton, CT). FTIR analysis was also used to determine the molar composition of NIPAAm and AAm in the samples, using ratios of characteristic peak heights for each monomer.

### Gel permeation chromatography

The molecular weights of NIPAAm-*co*-AAm oligomers were determined using GPC (System controller SCL-10A VP and Liquid controller LC-10AT VP, Shimadzu Scientific Instruments, Norcross, GA). To calibrate the instrument, approximately 0.002 g of polystyrene molecular weight standards (Aldrich Chemical Company) were dissolved in 1 mL high-performance liquid chromatography-grade THF, filtered through a 0.4 μm-size filter (Osmonics, Minnetonka, MN), and introduced along with the mobile-phase THF into Styragel® HR 1 (molecular weight range 100–5000) and Styragel HR 4 (molecular weight range 5000–100,000) columns (7.8 mm × 300 mm; Waters, Milford, MA) connected in series. The flow rate of the mobile phase was set between 0.5 and 1.0 mL/min (the maximum rate allowable that would ensure a pressure drop lower than 3.5 psi), and eluted polymers were detected by a differential refractometer (Shimadzu, RID 10A). The retention times were converted to molecular weights using a calibration plot for the polystyrene standards. The columns were calibrated each time before a series of oligomer samples were run to eliminate differences in flow rate or column properties.

### UV–Vis turbidimetry

To determine the transition temperature and the response time for the oligomers when exposed to a change in temperature, turbidity experiments were performed on the aqueous oligomer solutions (7 mg/mL), which were heated in thermally jacketed cuvettes inside an UV–Vis spectrophotometer (Shimadzu Scientific Instruments; 1650PC). Absorbance data were collected during both heating and cooling experiments. For the heating experiments, the water jacket surrounding the sample cuvettes was changed from room temperature to 60°C. This caused a gradual heating of the oligomer samples to about 55°C, during which the temperature inside the cuvette was measured with a thermocouple and absorbance was measured at 500 nm every 2.5 s. Cooling experiments were done by starting with the water jacket at 60°C and changing to a water source kept at 5°C, with similar absorbance and temperature readings taken during the experiment.

**TABLE I**
**LCSTs for P(NIPAAm-*co*-AAm) Hydrogels as Determined by Equilibrium Swelling**

| Hydrogel | LCST (°C) |
|---|---|
| P(NIPAAm) | 34 |
| P(NIPAAm-*co*-9.2% AAm) | 38 |
| P(NIPAAm-*co*-13.1% AAm) | 41 |
| P(NIPAAm-*co*-16.7% AAm) | 42 |
| P(NIPAAm-*co*-20% AAm) | 42 |

### Mechanical analysis

Mechanical tests were used to collect data to estimate the mesh size of PHEMA and grafted PHEMA samples. Mechanical testing was carried out on equilibrium-swollen hydrogel samples using an Instron mechanical testing apparatus (Model 5581, Instron Corp., Norwood, MA) with a BioPuls® temperature-controlled bath to enable testing at physiological and elevated temperatures. PHEMA gels that were equilibrated in water at 37°C and 47°C were cut into dog-bone shapes of length 2.54 cm and center width of 0.635 cm. They were clamped on both ends, lowered into the water bath that was pre-equilibrated at 37°C or 47°C, and an incremental tensile load of 5 kN was applied to stretch the sample at a constant strain rate of 1.27 cm/min until the sample broke.

## RESULTS AND DISCUSSION

### Synthesis and characterization of P(NIPAAm-*co*-AAm) hydrogels

P(NIPAAm-*co*-AAm) hydrogel samples were successfully synthesized. The hydrogel samples were transparent in color and uniform in appearance. Equilibrium swelling experiments showed the thermosensitive behavior of copolymers with varying comonomer content. The approximate LCST of each of the copolymer gels was determined from the onset point of transition from a polymer fraction versus temperature plot[20] for each of the gels (Table I), with LCSTs for P(NIPAAm-*co*-AAm) hydrogels ranging from about 34°C to 42°C. The increase in LCST caused by increasing the AAm content is consistent with other reports[6,11,21] and is thought to occur because an increase in the number of hydrophilic pendent groups (e.g., CONH for AAm) facilitates the formation of an increased number of hydrogen bonds (when equilibrated in water) stabilizing the swollen structure until higher temperatures are reached compared with homopolymers of NIPAAm.

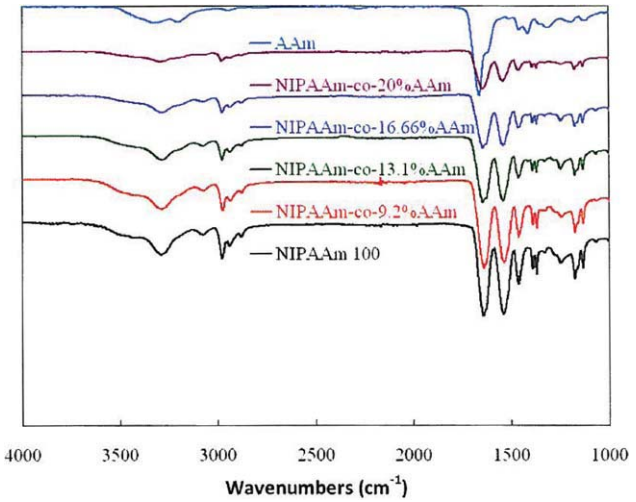


**Figure 3** FTIR spectra for AAm, NIPAAm, and (NIPAAm-*co*-AAm) oligomer samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

### Oligomer synthesis and characterization

Polymerization of (NIPAAm-*co*-AAm) oligomers was successfully carried out as verified by the appearance of a viscous opaque mass that settled to the bottom of the reaction vial. The molecular structures of dried oligomer samples of NIPAAm, AAm, and (NIPAAm-*co*-AAm) were characterized by FTIR. NIPAAm and AAm differ in their molecular structure in that AAm has a primary amide CO—$NH_2$, whereas NIPAAm has a secondary amide CO—NH—R, with R representing the isopropyl group. From Figure 3, which shows the FTIR spectra of these oligomers, the presence of AAm and NIPAAm in the oligomers was verified, whereas the intensities of the bands in the (NIPAAm-*co*-AAm) oligomer samples was used to determine the ratio of repeating units present in each sample. The wave numbers for each of the characteristic bonds in AAm and NIPAAm are listed in Table II. To determine the composition of the oligomers, the ratio of the characteristic absorption peaks in NIPAAm because of the isopropyl group appear at 1386 and 1367 $cm^{-1}$ to the intensity of the C=O peak at 1656 $cm^{-1}$ was calculated. A calibration curve was obtained by mixing the homopolymers of NIPAAm and AAm with varying molar ratios. The mole fractions of NIPAAm and AAm in the oligomers determined by this method are shown in Table III. The difference in monomer mole fractions between the feed and product indicates that the monomers had different reactivity ratios, with NIPAAm being incorporated at a somewhat higher level in most cases.

**TABLE II**
**Group Wave Numbers for Covalent Bonds in AAm and NIPAAm**

| Vibration | Wave number ($cm^{-1}$) |
|---|---|
| AAm | |
| H-N-H stretches | 3170 and 3356 (two bands) |
| C=O stretch (strong) | 1660 |
| $NH_2$ scissors | 1625 (shoulder) |
| C-N stretch | 1413 |
| NIPAAm | |
| NH stretch | 3300 |
| C=O stretch (strong) | 1656 |
| NH in plane bend (strong) | 1541 |
| C-N stretch | 1250 |
| Isopropyl band | 1386 and 1367 |

**TABLE III**
**Compositions of NIPAAm and AAm in the Feed and in the Copolymer, as Determined by Infrared Spectroscopy**

| Mole fraction of NIPAAm in feed $m_1$ | Mole fraction of AAm in feed $m_2$ | Mole fraction of NIPAAm in copolymer $M_1$ | Mole fraction of AAm in copolymer $M_2$ |
|---|---|---|---|
| 90.8 | 9.2 | 98.2 | 1.8 |
| 86.9 | 13.1 | 87.2 | 12.8 |
| 83.3 | 16.7 | 82 | 18.0 |
| 80 | 20 | 70.9 | 29.1 |
| 70 | 30 | 82.4 | 17.5 |
| 60 | 40 | 70.1 | 29.0 |

### Oligomer molecular weights

Oligomer molecular weight distributions and polydispersity indices obtained from GPC are reported in Table IV. With an increase in AAm composition, there was a slight decrease in weight-average molecular weight with the (NIPAAm-*co*-20%AAm) oligomer sample having the lowest molecular weight of 3840, whereas the oligomers containing 9.2, 13.1, and 16.7% AAm had molecular weights on the order of 5000. The decrease in molecular weight with increase in AAm content could be due to the lower reactivity ratio of AAm compared with that of NIPAAm. Thus, as the feed ratio of NIPAAm decreased, the molecular weight also decreased. The difference in molecular weight and structure between cross-linked polymers and low-molecular-weight noncross-linked oligomers can influence thermodynamic and kinetic properties such as the

**TABLE IV**
**Molecular Weights of P(NIPAAm-*co*-AAm) Oligomers with Respect to Polystyrene Standards**

| AAm content in comonomer feed (%) | $\overline{M}_w$ | $\overline{M}_n$ | PDI |
|---|---|---|---|
| 9.2 | 5580 | 3430 | 1.63 |
| 13.1 | 5440 | 3440 | 1.67 |
| 16.7 | 5400 | 3140 | 1.72 |
| 20.0 | 3840 | 2320 | 1.65 |

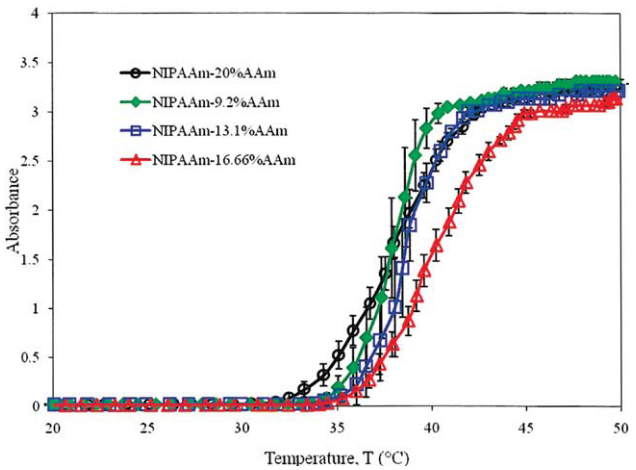


**Figure 4** Absorbance of (NIPAAm-*co*-AAm) oligomers containing 9.2, 13.1, 16.7, and 20 mol % AAm as a function of temperature at 500 nm. Samples were heated at 4.2°C/min from 20°C to 50°C, and absorbance values were recorded simultaneously. Error bars indicate standard deviation for three different runs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

phase-transition temperature and the response times of thermoresponsive materials.

### Oligomer LCSTs

Using equilibrium turbidity analysis, (NIPAAm-*co*-AAm) oligomers were found to have LCSTs in a desirable range, above physiological temperature (Fig. 4). As the oligomers phase separate from water, the solutions become turbid. Thus, the approximate LCST of each of the oligomer samples was determined from the temperature where the absorbance was 50% of maximum (Table V), as indicated by the midpoint of the thermal transitions. As was observed with swelling data for P(NIPAAm-*co*-AAm) hydrogels, an increase in AAm content in the oligomers increased the LCST. With the increase in AAm content from 9.2 mol % to 13.1–16.7 mol %, the LCST of the oligomer increased; however, a further increase in AAm content to 20 mol % caused a drop in the LCST. Because the oligomer compositions were set in the initial part of the polymerization reaction, the monomer feed concentrations, though the same as those for some of the P(NIPAAm-*co*-AAm) hydrogels, result in materials with different NIPAAm : AAm ratios and have different phase-transition temperatures. Other factors such as the presence of hydrophilic cross-linking agents EGDMA or MBAAm that render the hydrogel more hydrophilic or the increased importance of the initiator molecules in the oligomer structure could explain why the LCSTs of hydrogels are larger than those of oligomers. In either case, the phase-transition temperatures even in small-molecular-weight oligomers were successfully tuned by varying the monomer composition. The oligomer LCSTs determined from the turbidity study allow for the selection of compositions to use in the design of grafted PHEMA networks that can be activated to release drugs when heated above 37°C.

Turbidity responses were measured for the oligomer solutions when exposed to a temperature change from 20°C to 54°C (Fig. 5). It was observed that the change in absorbance values for the oligomer solutions with change in temperature was almost instantaneous on crossing the LCST. This parameter provides an estimate of how fast the oligomers grafted to the PHEMA network would respond by expanding or collapsing to close or open mesh space for diffusion with a change in temperature. This information can further be used while designing the drug-delivery system with an on/off response to temperature.

From a separate oligomer turbidity study, the response of oligomer solutions subjected to repeated heat–cool cycles was obtained. The rates of heating and cooling were calculated from the slope of the temperature versus time data using a line drawn through temperature data points between the low steady-state temperature, 20°C (or high steady-state temperature 54°C for cooling rate) to the corresponding LCST temperature (Table VI). In cases where the heating and cooling rates matched (e.g., the first cooling and third heating rates for (NIPAAm-*co*-9.2%AAm) oligomers were both 3.6°C/min), the LCSTs obtained during the cooling procedure were always lower than the ones obtained during the heating process. This is because, when the samples are heated to temperature above LCST, some additional intersegment hydrogen bonds are formed between the collapsed chains; thus, during cooling this chain, dissociation occurs only when the temperature is lower than the LCST. Similar observations have been made by several researchers on NIPAAm-based polymers;[22–28] Cheng et al.[25] confirmed the presence of additional hydrogen bonds during polymer heating by FTIR analysis where the intensity for hydrogen bonding between polymer

**TABLE V**
**LCSTs Calculated from Turbidity Data for (NIPAAm-*co*-AAm) Oligomer Samples**

| AAm content in comonomer feed (%) | LCST (°C) |
|---|---|
| 9.2 | 37.5 |
| 13.1 | 38.0 |
| 16.7 | 40.0 |
| 20.0 | 38.0 |

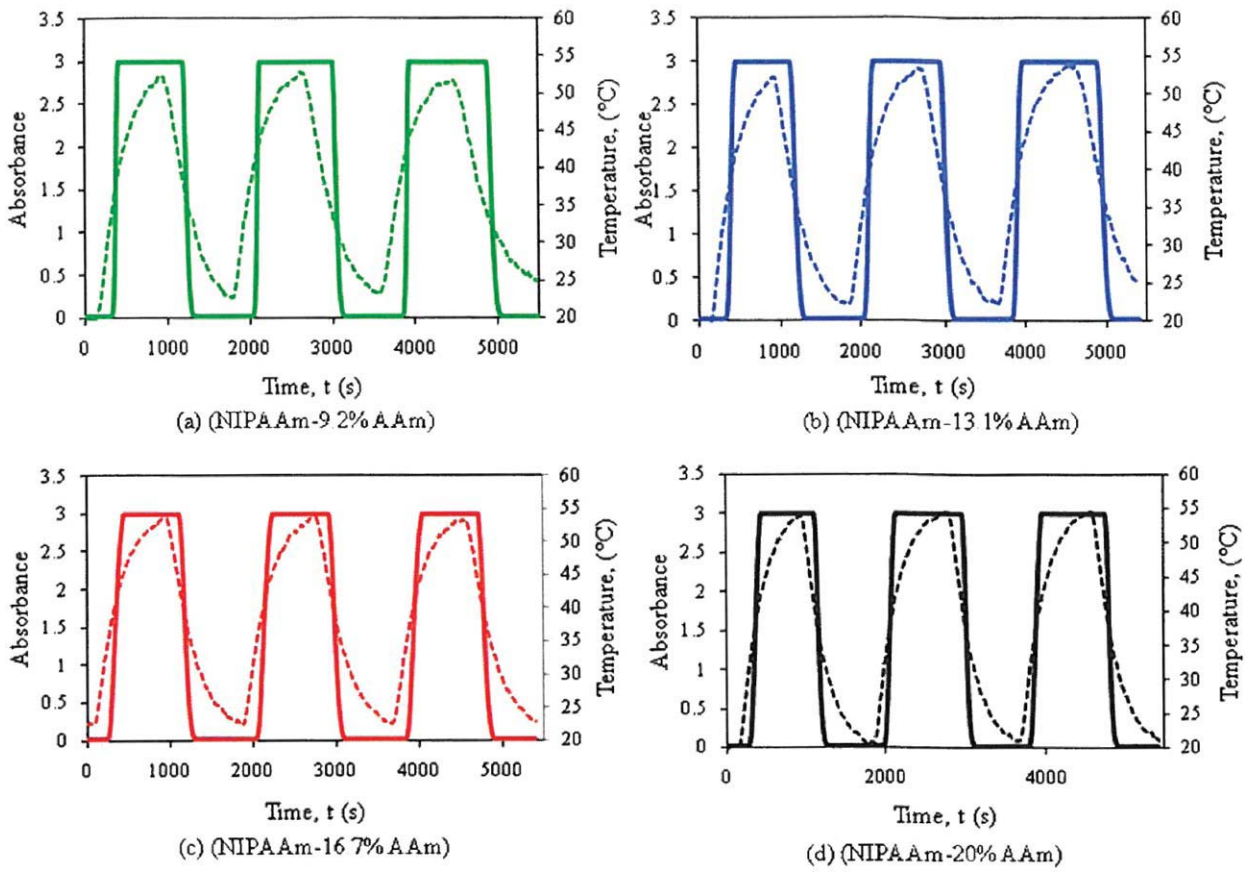


**Figure 5** Thermally induced phase behavior (turbidity) as measured by the absorbance of (NIPAAm-*co*-AAm) oligomers as a function of time and temperature at 500 nm. Solid lines indicate absorbance; dotted lines indicate temperature. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

chains was higher when solution temperature increased above the LCST.

### PHEMA and grafted P(HEMA-*g*-(NIPAAm-*co*-AAm)) hydrogel synthesis

PHEMA samples, which are solid and transparent, were successfully synthesized in the method discussed in the Experimental section. In the highly cross-linked PHEMA hydrogels, an increase in cross-linking density created samples that were more rigid and had lower swelling ratios. For these higher cross-link densities, those that were cross-linked with MBAAm cracked after polymerization; thus, EGDMA was preferred for the synthesis of PHEMA and grafted hydrogels.

**TABLE VI**
**LCSTs for (NIPAAm-*co*-AAm) Oligomer Samples Calculated from Turbidity Experiments Conducted through Three Heating/Cooling Cycles**

| AAm content in comonomer feed (%) | Cycle 1 | | Cycle 2 | | Cycle 3 | |
|---|---|---|---|---|---|---|
| | Heat | Cool | Heat | Cool | Heat | Cool |
| 9.2 | | | | | | |
| LCST (°C) | 38 | 36 | 42 | 32 | 42 | 32 |
| Heating rate (°C/min) | 4.8 | −3.6 | 4.2 | −3.0 | 3.6 | −2.4 |
| 13.1 | | | | | | |
| LCST (°C) | 38 | 36 | 38 | 36 | 38 | 37 |
| Heating rate (°C/min) | 4.8 | −3.6 | 4.2 | −3.6 | 4.8 | −3.0 |
| 16.7 | | | | | | |
| LCST (°C) | 40 | 38 | 40 | 38 | 40 | 38 |
| Heating rate (°C/min) | 4.8 | −4.2 | 4.8 | −4.2 | 4.2 | −3.6 |
| 20 | | | | | | |
| LCST (°C) | 38 | 36 | 38 | 36 | 38 | 36 |
| Heating rate (°C/min) | 5.4 | −4.2 | 5.4 | −4.2 | 5.4 | −4.2 |

**TABLE VII**
**Tensile Moduli Obtained from Tensile Stress–Strain Data for PHEMA Gels Cross-linked with Increasing Amounts of EGDMA and MBAAm at 37°C and 47°C**

| Cross-linking agent | Nominal cross-linking density (mol %) | Tensile modulus at 37°C (MPa) | Tensile modulus at 45°C (MPa) |
|---|---|---|---|
| EGDMA | 1.0 | 2.29 ± 0.36 | 1.85 ± 0.133 |
| EGDMA | 5.0 | 6.30 ± 0.87 | 6.21 ± 0.87 |
| EGDMA | 10.0 | 12.70 ± 0.59 | 11.86 ± 0.64 |
| EGDMA | 12.0 | 23.35 ± 0.43 | 18.09 ± 0.39 |
| EGDMA | 13.5 | 30.66 ± 1.00 | 22.83 ± 1.37 |
| EGDMA | 15.0 | 42.10 ± 1.51 | 33.26 ± 4.22 |
| MBAAm | 1.0 | 0.78 ± 0.06 | 0.58 ± 0.16 |
| MBAAm | 5.0 | 2.24 ± 0.11 | 2.05 ± 0.21 |
| MBAAm | 10.0 | 4.27 ± 1.04 | 5.15 ± 0.15 |

Error indicates standard deviation for three samples.

As discussed in the Experimental section, aminoethane thiol-terminated oligomers were activated using acryloyl chloride before the grafting reaction as shown in Figure 2. Grafted P(HEMA-*g*-(NIPAAm-*co*-AAm)) hydrogels were successfully synthesized. The samples were solid and transparent and seemed to be more fragile than ungrafted PHEMA gels. They were rinsed and equilibrated in water. With an increase in temperature to 47°C, equilibrated grafted gels turned from being transparent to white because of the phase separation of the (NIPAAm-*co*-AAm) oligomers, which went from being colorless in their hydrophilic state to white in their hydrophobic state above their transition temperature.

### Characterizing hydrogel structure

Stress–strain data and equilibrium-swelling data were used to characterize the structure of hydrogels and determine the molecular weight between cross-links, $\overline{M}_c$, and the mesh size, $\xi$, available for diffusion of imbedded solutes or drugs through PHEMA and grafted gels cross-linked with either MBAAm or EGDMA at concentrations of 1, 5, 10, 12, 13.5, and 15 mol %. Rubber-elasticity and equilibrium-swelling data were used to calculate $\overline{M}_c$ in the PHEMA network. Using rubber elasticity theory,[29] $\overline{M}_c$ was determined from mechanical stress experiments through:

$$E\nu_{2,s}^{-1/3} = RT\rho_{2,r}\left(\frac{1}{M_c} - \frac{2}{M_n}\right)\left(\frac{\nu_{2,s}}{\nu_{2,r}}\right)^{1/3} \quad (2)$$

In this equation, $E$ is the tensile modulus of each PHEMA or P(HEMA-*g*-(NIPAAm-*co*-AAm)) sample (in $N/m^2$) obtained by mechanical testing in aqueous solutions at 37°C or 47°C, $R$ is the universal gas constant, $T$ is absolute temperature, and $\rho_{2,r}$ is the density of the hydrogel in the relaxed or as-formed state. The parameters $\upsilon_{2,r}$ and $\upsilon_{2,s}$ are the polymer fractions in freshly made relaxed and equilibrium swollen states, respectively, and were determined by a buoyancy method.[30] $\overline{M}_n$ is the number-average linear molecular weight of the polymer chains. The value of $\overline{M}_c$ was then used to calculate the mesh size, $\xi$, of the sample by:

$$\xi = (\nu_{2,s})^{-1/3}(r_o^2)^{1/2} \quad (3)$$

Here, $r_0$, the end-to-end length of the polymer segment between two consecutive cross-links, is given by:

$$(r_o^2)^{1/2} = \left(\frac{2\overline{M}_c}{M_r}\right)^{1/2} C_n^{1/2} l \quad (4)$$

where $M_r$ is the molecular weight of a single repeating unit (130.4 for PHEMA), and $C_n$ is the characteristic ratio of PHEMA in water which was determined as 6.9 by Dušek and Sedláček.[31] The characteristic ratio is defined as the ratio of the mean square unperturbed end-to-end distance for a real chain (or a model of a chain) to the value expected for a freely joined chain with the same number of bonds, of the same mean square length. $l$ is the distance between two covalently-linked backbone carbon atoms (1.54 Å).

The mesh space calculation allows for a selection of polymer structures needed to accommodate the release of a particular drug. With an increase in the number of cross-links in the network, the mobility of chains decreases and the capacity of the gel to swell is reduced, rendering a more rigid structure. Thus, the network becomes tighter and the mesh space enclosed within the network chains becomes smaller with increasing cross-link density. Table VII lists the tensile moduli (slope of the elastic region of the stress–strain curves) of PHEMA gels cross-linked with varying concentrations of EGDMA and MBAAm. The elastic moduli of gels cross-linked with MBAAm are smaller than those cross-linked with EGDMA. This is because of the two hydrophilic AAm groups in MBAAm, which increase the hydrophilicity of the network, thus increasing the capacity to swell, which was indicated by larger swelling ratios for MBAAm-cross-linked hydrogels. With increase in temperature to 45°C, the modulus decreased because of the increase in chain mobility with increase in temperature due to chain relaxation. Increased temperatures also improve polymer–solvent interactions, thus increasing the swelling capacity (although only to a small extent), thereby increasing elasticity. Mechanical testing to calculate mesh sizes for grafted gels was also performed, with

the grafted gels being much more fragile than the ungrafted gels as confirmed by the lower tensile moduli. The fragility of the grafted gels is attributed to a combination of increased network hydrophilicity because of the presence of the grafts and decreased cross-linking effectiveness during the cross-linking polymerization reaction.

The mesh sizes of PHEMA cross-linked with increasing concentrations of EGDMA and MBAAm were calculated using rubber elasticity theory at 37°C and 45°C (Figs. 6 and 7). Both plots indicate that mesh size decreases with an increase in cross-linking density as was expected. Also, an increase in temperature increased the mesh size to a small extent. Gels cross-linked with MBAAm, which has two hydrophilic AAm groups, had higher mesh sizes than those cross-linked with EGDMA, confirming studies conducted by Mudassir and Ranjha[32] and Karadağ and Saraydin[33] that showed MBAAm-cross-linked hydrogels have larger swelling fractions and consequently larger mesh sizes than EGDMA-cross-linked gels of the same composition. Because of lower tensile moduli and larger swelling fractions compared with ungrafted gels, the calculated mesh sizes of grafted gels were slightly larger than those of ungrafted gels.

## CONCLUSIONS

Thermoresponsive (NIPAAm-*co*-AAm) oligomers with varying compositions of NIPAAm and AAm were synthesized by free-radical polymerization and characterized by GPC, FTIR, and UV-vis turbidimetry and were used to make P(HEMA-*g*-(NIPAAm-*co*-AAm)) hydrogels. Oligomers with varying compositions and chain lengths exhibited differences in LCSTs and rate of response to temperature. Thus, oligomers with desired structures, chain lengths, and tunable properties can be synthesized for applications in several fields.

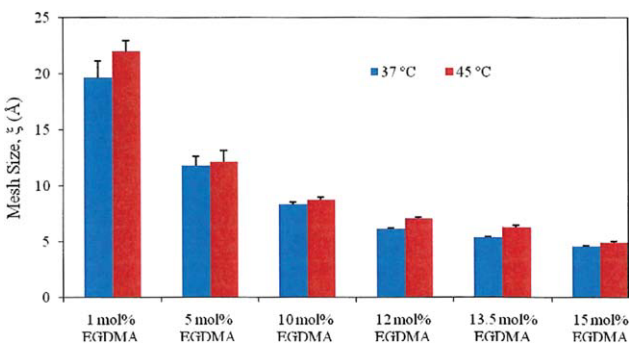


**Figure 6** Mesh sizes for PHEMA cross-linked with EGDMA at 37°C and 45°C calculated from rubber-elasticity theory and equilibrium-swelling data. Error bars indicate standard deviation for three samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

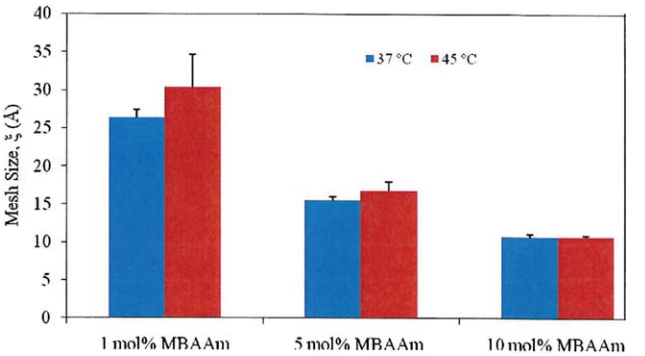


**Figure 7** Mesh sizes for PHEMA cross-linked with MBAAm at 37°C and 45°C calculated from rubber-elasticity theory and equilibrium-swelling data. Error bars indicate standard deviation for three samples. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Tensile moduli and mesh sizes of the hydrogels varied with the amount and type of cross-linking agent used. MBAAm-cross-linked PHEMA gels had larger mesh sizes than EGDMA-cross-linked gels. Increase in cross-link density decreased mesh sizes as was expected. Temperature had a slight effect on mesh size, as the measured tensile moduli decreased with the increased polymer–solvent interaction with an increase in temperature. Further studies to determine the effect of graft length, graft density, and graft composition on the performance of the grafted system as a drug delivery device are discussed in a subsequent article.

Grafted hydrogel systems with grafted oligomers described in this article would be beneficial for the development of hyperthermia-activated drug release, as is currently in development for the localized combination therapy treatment of cancers.[34] Because PHEMA and P(NIPAAm-*co*-AAm) do not have ionizable groups, they are expected to have similar thermal behavior in biological solutions that is comparable to the results found here for tests conducted in deionized water. Thus, the oligomers developed here have great potential for use in heating-activated drug delivery.

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