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# *N*-isopropylacrylamide-based Copolymers with Time-dependent LCST for a Bioresorbable Carrier

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## ABSTRACT

To develop a new class of *in situ*-forming, injectable, and biodegradable polymeric biomaterials based on time-dependent lower critical solution temperature (LCST) properties for localized delivery, copolymers of N-isopropylacrylamide (NIPAAm), 2-hydroxyethyl methacryl lactate (HEMA-lactate) and acrylic acid (AAc) were prepared with varying mole ratios of monomers. The copolymers showed LCST and gelation properties below body temperature in 0.1 N PBS solution of pH 7.4. The LCST and gelation temperature of the copolymers decreased as the HEMA-lactate content of the copolymers was increased. The copolymers also showed time-dependent LCST and gelation properties in 0.1 N PBS solution of pH 7.4 owing to hydrolysis of HEMA-lactate. Hydrolysis of HEMA-lactate caused the polymers to be more hydrophilic, resulting in an increase in LCST and gelation temperature. All the polymers with about 6 mol % AAc exhibited LCST and gelation temperature above body temperature after complete hydrolysis of HEMA-lactate.

## INTRODUCTION

Poly(NIPAAm) based materials with biodegradable and thermosensitive properties have been studied for biomedical applications such as tissue engineering and drug delivery [1-5]. Typical examples are as follows: a N-isopropylacrylamide copolymer crosslinked with biodegradable poly(amino acids) [1]; temperature responsive and biodegradable poly(N-isopropylacrylamide-co-caprolactone) [2]; partially degradable dextran–maleic anhydride/ poly(N-isopropylacrylamide) hybrid hydrogels [3]. Also, Nevadovic et al. designed new types of thermosensitive NIPAAm-based polymers such as a copolymer of NIPAAm and 2-hydroxyethyl methacryl lactate (HEMA-lactate) [4], a copolymer of NIPAAm and N-(2-hydroxypropyl)methacrylamide lactate (HPMAm-lactate), and a copolymer of NIPAAm, HPMAm-lactate and PEG (poly(ethylene glycol)) [5]. Especially, the poly(NIPAAm-co-HPMAm)-b-PEG formed micelles and cmt (critical micelle temperature) was below body temperature. The cmt of poly(NIPAAm-co-HPMAm-lactate)-b-PEG block copolymers with 35 and 50 % HPMAm-lactate reached values above 37 °C after hydrolysis. In the present study, copolymers with gelation properties as well as with biodegradability and LCST were designed by introducing acrylic acid into copolymers of NIPAAm and HEMA-lactate.

## EXPERIMENTAL

N-isopropylacrylamide (Aldrich) was purified by recrystallization from n-hexane and dried under vacuum for four days. Acrylic acid (Aldrich) was distilled at 39°C/10mmHg. 2,2'-Azobisisobutyronitrile (AIBN; Aldrich research chemicals (U.K) 98 %) was purified by recrystallization from methanol. 2-Hydroxyethyl methacrylate (HEMA; Avocado, 98 %), lactide ((3*S-cis*)-3,6-dimethyl-1,4-dioxane-2,5-dione, Aldrich 98 %) and stannous 2-ethylhexanoate ($SnOct_2$, Aldrich) were used as received.

The molecular weights of the synthesized polymers were determined by gel permeation chromatography (Shimadzu HPLC) in conjunction with static light scattering (Wyatt miniDawn® -Santa Barbara, CA). The content of AAc was determined by titration. Each copolymer (0.1 g) was dissolved in 20 ml of deionized water by cooling at 4 °C and the solution was brought to room temperature and then titrated with 0.01N NaOH using phenolphthalein as an indicator. Differential scanning calorimetry was used to estimate the lower critical solution Temperatures (LCST) of all the copolymers. Each copolymer was dissolved in 0.1M Phosphate Buffered Saline (PBS) at 5-wt %. The solution pH was adjusted to 7.4 with 0.1 N NaOH and 0.500 ± 0.005 grams of the solution was placed in DSC ampoules and then tested in triplicate from 0 to 80 °C at 1 °C/min. PBS (0.1 M) was used for the baseline.

Quantification of the elastic or storage modulus for these materials was investigated using rheometry. Polymer solutions (1.26 ml, 21 wt %) were placed between the parallel plates of 40 mm diameter and a gap of 1.0 mm at various temperatures (22 and 37 °C) The heating rate was 1 °C/min. The sol to gel transition temperature was defined as the temperature at which loss modulus (G") is equal to storage modulus (G').

Complete hydrolysis was carried out as follows: Five wt % solutions of poly(NIPAAm-co-HEMA-lactate-co-AAc) in 0.1 M PBS were prepared and adjusted to pH 10.5, using 1 NaOH on a daily basis. The samples were incubated in a water bath at 37 °C for 5 days and then dialyzed in distilled water. The samples were then lyophilized and then adjusted to pH 7.4 prior to LCST measurements.

### Synthesis of poly(NIPAAm-co-HEMA-lactate-co-AAc)

HEMA-lactate was synthesized using HEMA and l-lactide according to the literature previously reported [4]. The average degree of polymerization (dp) of HEMA-lactate obtained was 2.3 and 2.5. Purity was more than 93 %. Copolymers were prepared as described below. The NIPAAm/HEMA-lactate /AAc ratios were 84/10/6, 79/15/6, and 74/20/6 (mol/mol/mol), respectively. The total monomer concentration was 0.1 g/mL in 1, 4-dioxane (10 wt %). AIBN was used as initiator (initiator / total amount of monomers = $7 \times 10^{-3}$ mol/mol). Approximately, 10 g batches were prepared. Nitrogen was bubbled through the solution at room temperature for 15 minutes prior to addition of the initiator (AIBN) to reduce oxygen content in the polymerization reaction. The co-polymerization was conducted at 65 °C for 20 h in a nitrogen atmosphere. Subsequently, the solvent was removed under reduced pressure and the copolymers were dissolved in acetone (around 20% (w/v)) and precipitated in an excess of diethyl ether. The precipitated polymers were isolated by filtration and dried in a vacuum oven at 40°C.

## RESULTS AND DISCUSSION

A list of the copolymers synthesized is found in Table 1. The mole ratio of NIPAAm and HEMA-lactate was calculated from the integration ratio between the methyl proton (6H) of NIPAAm and the methine proton (1H) of HEMA-lactate appearing at 1.1 and 5.3 ppm, respectively. The actual content of AAc was determined for each sample by acid titration. All copolymers are soluble below LCST in aqueous solution as well as in organic solvents such as chloroform, THF, dioxane, and acetone. The polymers were found to have weight-average molecular weights in the range (1.6-7.8) x $10^5$, as seen in Table 1.

**Table 1.** Characteristics of the poly(NIPAAm-HEMA-lactate-co-AAc) copolymers.

| N | Copolymers | | | LCST (°C) | | Gel Temperature (°C) | MW (x$10^{-5}$) |
|---|---|---|---|---|---|---|---|
| | NIPAAm (mol %) | HEMA –lactate (mol %) | Acrylic Acid (mol %) | Before Hydrolysis | After Hydrolysis | | |
| 1 | 84.4 | 9.4 (dp=2.3) | 6.2 ± 0.1 | 34.9 ± 0.2 | 44.1 ± 0.2 | 34 | 2.3 |
| 2 | 81.7 | 12.3 (dp=2.3) | 6.0 ± 0.1 | 28.4 ± 0.1 | 44.0 ± 0.1 | 28 | 2.4 |
| 3 | 75.8 | 18.5 (dp=2.3) | 5.7± 0.1 | 23.5 ± 0.9 | 43.2 ± 0.1 | 24 | 1.6 |
| 4 | 80.8 | 13.5 (dp=2.5) | 5.8 ± 0.1 | 28.5 ± 0.2 | 43.3 ± 0.1 | 24 | 7.8 |

### Thermosensitivity

NIPAAm is a representative thermosensitive polymer with LCST at around 32 °C. The LCST of NIPAAm-based copolymers is largely affected by comonomers. Acrylic acid is a monomer that has been used to raise the LCST and increase swelling [6]. So we introduced an anionic monomer (acrylic acid) to the poly (NIPAAm-co-HEMA-lactide) system. We synthesized poly (NIPAAm-co-HEMA-lactate-co-Acrylic Acid) by varying mole ratios of NIPAAm and HEMA-lactate with 6-mol % of acrylic acid fixed. As seen in Table 1, all the copolymers showed LCST values below body temperature. The LCST of the copolymers decreased as the content of the hydrophobic HEMA-lactate was increased: As the content of HEMA-lactate (dp=2.3) increased in the polymer order **1** (9.4 mol %) < **2** (12.3 mol %) < **3** (18.5 mol %), the LCST values of copolymers decreased in the polymer order **1** (34.9 °C) > **2** (28.4 °C) > **3** (23.5 °C) in the buffer solution of pH 7.4. Also, these copolymers exhibited gelation properties beyond a sufficient concentration. The gelation temperature of the copolymer solutions (21 wt %) decreased with raising the content of the hydrophobic HEMA-lactate. The content of HEMA-lactate increased in the polymer order **1** (9.4 mol %) < **2** (12.3 mol %) < **3** (18.5 mol %); the gelation point of polymers decreased in the polymer order **1** (34 °C) > **2** (28 °C) > **3** (24 °C) in the buffer solution of pH 7.4.

The rheological properties of the copolymers were studied at a polymer concentration of 21-wt %. Figure 1 shows the frequency dependence of the dynamic moduli for polymer **2** at 22 and 37 °C.

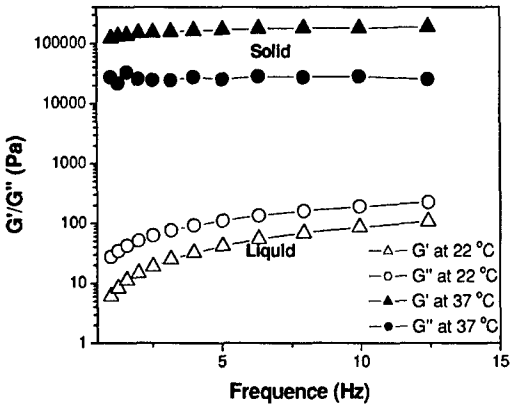


**Figure 1.** The frequency dependence of the dynamic moduli for copolymer **2** at 22 and 37 °C.

At 22 °C, the loss modulus (G") of copolymer **2** was larger than the storage modulus (G') in the frequency range. On the other hand, the storage modulus (G') was higher that the loss modulus (G") at 37 °C. This result indicated that the polymer solution was in a sol state at 22 °C and underwent a transition from a viscoelastic fluid to an elastic solid at body temperature.

## Hydrolysis

The LCST values of the copolymers were below body temperature as seen in Table 1. After complete hydrolysis, the LCST values of the copolymers, however, were above body temperature: The LCST values of copolymers **1**, **2**, **3**, and **4** were 44.1, 44.0, 43.2 and 43.3, respectively after hydrolysis. This indicated that poly(NIPAAm-co-HEMA-lactate-AAc) was converted into poly(NIPAAm-co-HEMA-AAc) after hydrolysis. Conversion of HEMA-lactate to HEMA made the polymer more hydrophilic.

Figure 2 photographically demonstrates the sol-gel transition of copolymer **4**. Copolymer **4** was dissolved on the concentration of 15-wt % in 0.1 M PBS solution of pH 7.4. As it was taken out of the cold chamber at 4 °C, the polymer solution was in a transparent sol state as shown in figure 2 (a). In contrast to this, after the temperature jumped from 4 to 37 °C, the polymer solution became a turbid gel state (b). After 1-day incubation at 37 °C, the copolymer gel became a shrunken gel (c). The shrunken gel gradually transitioned into a transparent sol after 6 days, indicating that the LCST of the copolymer increased above 37 °C due to hydrolysis of HEMA-

lactate. This result might suggest that these gels can be eliminated by the dissolution of the polymers after hydrolysis under physiological condition.

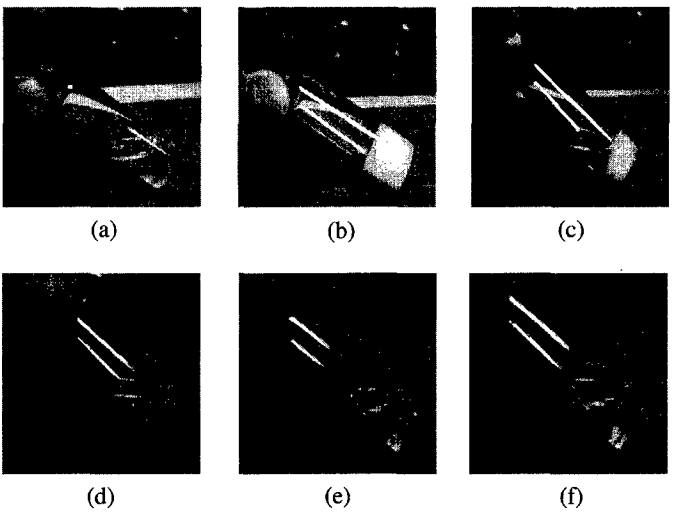


**Figure 2.** Demonstration photographs of the sol-gel transition of copolymer **4** at 15 wt % solution in 0.1 M PBS of pH 7.4 during the incubation of 6 days at 37 °C: (a) at 4 °C, (b) at 37 °C, (c) after 1 day at 37 °C, (d) after 2 days at 37 °C, (e) after 4 days at 37 °C, (f) after 6 days at 37 °C.

## CONCLUSIONS

Poly(NIPAAm-co-HEMA-lactate-co-AAc) copolymers were synthesized with varying mole ratios of monomers. All copolymers had LCST and gelation properties in aqueous solution. The LCST values and gelation temperature of the copolymers decreased with increasing a mole ratio of hydrophobic HEMA-lactate part on copolymers. The copolymers have time-dependent LCST properties in 0.1 N PBS solution of pH 7.4 owing to hydrolysis of HEMA-lactate. Hydrolysis of HEMA-lactate caused the polymers to be more hydrophilic, resulting in the increase in LCST. After hydrolysis all the polymers exhibited LCSTs above body temperature and lost gel properties at body temperature. These copolymers would be useful for a bioresorbable drug carrier.

## ACKNOWLEDGMENTS

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## REFERENCES


1. Yoshida T, Aoyagi T, Kokufuta E, Okanno T, *J Polym Sci Part A: Polym Chem* **41**, 779 (2003)
2. Sun L-F, Zhuo R-X, Liu Z-L, *Macromol Biosci* **3**, 726 (2003)
3. Zhang X, Wu D, Chu C-C, *Biomaterials* **25**, 4719 (2004)
4. Neradovic D, Hinrichs WLJ, Kettenes-van den Bosch JJ, Hennink WE, *Macromol. Rapid Commun.* **20**, 577 (1999)
5. Neradovic D, van Nostrum CF, Hennink WE, *Macromolecules* **34**, 7589 (2001)
6. Vernon B, Kim SW, Bae YH, *J. Biomed. Mater. Res.* **51**, 69 (2000)