

N-isopropylacrylamide-based Copolymer with Hydrolysis-dependent Lower Critical Solution Temperature as Injectable, Bioresorbable Gelling Materials for Controlled Drug Delivery

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Statement of Purpose: Temperature-sensitive poly(N-isopropylacrylamide) (poly(NIPAAm)) and its copolymers have been of high research interest for biomaterials applications, such as cell immobilization, drug delivery, etc. The low critical solution temperature (LCST) of poly(NIPAAm) copolymers can be controlled by varying the type and content of the co-monomers. Incorporation of hydrophobic monomers leads to a lower LCST and hydrophilic monomers to a higher LCST. Neradovic et al¹. reported the synthesis of a new type of thermo-sensitive NIPAAm copolymer with hydrolysable lactate ester side groups with the increase in LCST after hydrolysis. Synthesis of NIPAAm with cyclic monomer, 2-methylene-1,3-dioxepane (MD), and its biodegradable properties have been described by Sun, et al². Here, a novel thermo-sensitive, biodegradable NIPAAm copolymer, poly(NIPAAm-co-dimethyl- γ -butyrolactone-co-acrylic acid) is proposed that would have a hydrolysis-dependent LCST due to the hydrolysis of the ester bonds in dimethyl- γ -butyrolactone.

Methods: All materials were reagent grade and obtained from Aldrich & Co. unless otherwise noted. NIPAAm was dissolved in hexanes (10g in 100mL at 40°C) and then re-crystallized at room temperature. (R)-(+)- α -Acryloyloxy- β , β -dimethyl- γ -butyrolactone (BL) was used as received. Acrylic acid (AA) was distilled using vacuum at 39°C/10mmHg. 2, 2'-Azobisisobutyronitrile (AIBN) was dissolved in methanol (1g/20mL) at room temperature and re-crystallized at -20°C. Anhydrous 1,4-Dioxane was used as polymerization solvent and was treated by molecular sieve to remove the dissolved water before use. HPLC grade Tetrahydrofuran (THF) was used as the mobile phase for Static Light Scattering (MiniDawn, Wyatt Technology Corporation)/High Performance Liquid Chromatography (HPLC) (Shimadzu Corporation). Phosphate Buffered Solution (PBS) of pH 7.4 was used as the solvent for multi-cell Differential Scanning Calorimetry (DSC) (Calorimetry Science Corporation).

Poly(NIPAAm-co-dimethyl- γ -butyrolactone-co-acrylic acid) was synthesized in 1,4-dioxane at 65°C for 16 hours by radical polymerization. AIBN (7×10^{-3} mol AIBN/mol monomer) was used as the initiator for all reactions. The reaction was bubbled with Nitrogen (N₂) for 15 min. before adding the initiator and then maintained under a nitrogen environment throughout the reaction to eliminate oxygen. After polymerization, the solution was filtered and the solvent was evaporated. Then, the copolymer was dissolved in acetone and collected by precipitation in 14 to 15-fold-excess of diethyl ether. Finally, the copolymer was vacuum-dried for one day. Properties of the copolymer were characterized by DSC, HPLC, FTIR, NMR and acidic titration.

Results / Discussion: The copolymers showed decreased LCST from 35.23°C to 27.8°C with the increase in butyrolactone content. A time-dependent hydrolysis study was conducted in 0.1N PBS solution of pH 7.4 at 70°C and it showed that the LCST increases for each butyrolactone content with time. This increase resulted from the change of the hydrophobic butyrolactone ring to a more hydrophilic structure after hydrolysis. FTIR was conducted for on the degradation product during time-dependent hydrolysis. This indicated that the copolymer hydrolyzed with time. Two peaks at 1785 cm⁻¹ and 1750 cm⁻¹ were found in the FTIR spectra for the copolymers before hydrolysis and these two peaks are assigned to the two carbonyl groups in butyrolactone. With hydrolysis time, the peak at 1785 cm⁻¹ gradually disappeared and the peak at 1750 cm⁻¹ shifted to 1721 cm⁻¹. An NMR study showed that a peak at 5.3 ppm is from dimethyl- γ -butyrolactone and a peak at around 4.0 ppm from both dimethyl- γ -butyrolactone and NIPAAm. By comparing the LCST and hydrolysis process with previous studies, it was shown that the acrylic acid increased the LCST and accelerated the hydrolysis process. After hydrolysis for 10 days, the LCST increased 12.9°C to 25.2°C, depending on the content of dimethyl- γ -butyrolactone.

Table 1. Chemical Composition, Molecular Weight and LCST before and after Hydrolysis

	Chemical Composition (mol.%)			LCST(°C)	
	NIPAAm	BL	AA	Before	After 10d
1	92.72	3.26	4.02	35.23	48.13
2	90.60	5.76	3.63	31.37	49.97
3	88.37	8.12	3.51	27.80	53.00

Conclusions: Poly(NIPAAm-co-dimethyl- γ -butyrolactone-co-acrylic acid) was synthesized by radical polymerization. The initial LCST of the copolymer is between room temperature and body temperature. After 10 days' hydrolysis, the LCST increased above body temperature. These results indicated that poly(NIPAAm-co-dimethyl- γ -butyrolactone-co-acrylic acid) could be a potential biodegradable thermo-sensitive material for controlled drug delivery or tissue engineering applications, due to its LCST increase with hydrolysis time. Studies of the biocompatibility of these materials are underway.

References:

1. Dragana Neradovic, et. al, Macromol. Rapid Commun., 1999, (20: 577-581)
2. Li-Fang Sun, et. al, Macromol. Biosci, 2003, 3, (No.12: 725-728)