Thermo-Responsive PNIPAAm Copolymers with Hydrophobic Spacers

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Summary: We have synthesised and characterised three sets of acryloyl(amino-acid)/NIPAAm co-polymers. Three different co-monomers were synthesised and copolymerized with N-isopropylacrylamide (NIPAAm) by free radical polymerization in different molar ratios (12:1, 9:1, and 6:1). All series contain aliphatic spacers and are carboxylic terminated. The effect of co-monomer nature and content on the glass transition and the LCST transition in solution has been investigated. The main factor affecting the glass transition and the LCST of the polymer was found to be the side chain length of the co-monomer. The introduction of the co-monomer resulted in an overall increase in the polymer hydrophobicity which led to a decrease of both the polymer glass transition and the LCST transition observed in solution.

Keywords: copolymer; PNIPAAm; thermo-responsive

Introduction

Polymers displaying phase transitions in aqueous solution from a hydrophilic to hydrophobic state are of widespread interest as ‘smart’ biomedical materials.[1] The prototypical polymer in this class is poly(N-isopropylacrylamide) (PNIPAAm) and co-polymers derived from this basic material. One interesting feature of these polymers is that the lower critical solution transition (LCST) is observed in water around body temperature[2] i.e. 37 °C. These materials are currently being used in a variety of applications including cell attachment, drug release, separation membranes and tissue engineering.[3–6]

The LCST transition in PNIPAAm can be triggered using a variety of external stimuli such as temperature, pH and solvent composition. The two main effects responsible for the LCST are hydrophobic and hydrogen-bonding interactions. The exact mechanism and the role of each type of interaction are still debated in the literature.[13] The introduction of co-monomers in the polymer chain has a significant effect on the LCST; hydrophobic co-monomers lower the LCST while hydrophilic co-monomers increasing it.[ref]

In this work we have investigated the influence of hydrophobicity and co-monomer content on the LCST behaviour of a set of PNIPAAm based co-polymers. The generic chemical structures of NIPAAm and the co-monomers used are presented in Figure 1.

The co-monomers used are acidic and have hydrophobic spacer of varying length. Their hydrophobicity increases with increasing spacer length.

Experimental Part

Reagents and Solvents

All the reagents and solvents used for the synthesis and the characterisation of the co-monomers and co-polymers were purchased from Sigma Aldrich and used as received except for N-isopropylacrylamide (NIPAAm) and 4,4′-azoisobutyronitrile

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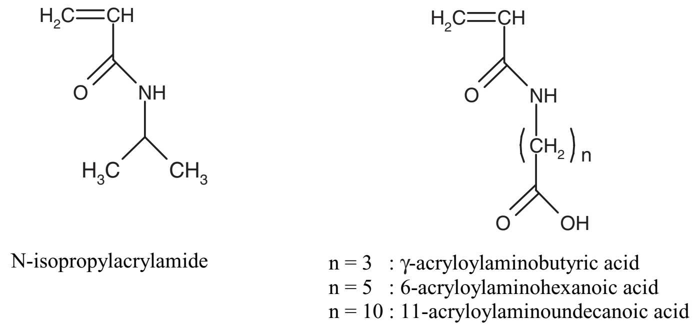


Figure 1.
Chemical structure of monomer and co-monomers used for the polymer synthesis.

(AIBN) which were purified by re-crystallisation from hexane and methanol respectively.

Synthesis of Co- Monomers

The co-monomers where synthesised as described by Kuckling et al.[7] following the synthetic route shown in Figure 2. The synthesis was carried out in a two-neck vessel under nitrogen atmosphere using the Schotten-Baumann reaction conditions.[8] The amino acids were first added to a NaOH water or ethanol solution depending on the amino acid used. The vessel was then cooled to 0 °C in an ice bath and acryloyl chloride added drop-wise while the suspension was stirred. The reaction mixture was further stirred for two hours. The residual solid phase was then removed by filtration and the solution acidified to pH ~ 1.5 by adding drop-wise concentrated HCl. The aqueous phase was extracted three times using ethyl acetate and the resulting organic phase was washed with deionised water in order to eliminate the salt that formed as a secondary product of the reaction. Water was then removed from the system using anhydrous calcium chloride, $CaCl_2$. The solution was filtered and the solvent finally removed using a rotary evaporator while the vessel was cooled in ice-cold water. The co-monomers were obtained in the form of white-yellow powders. The reaction conversion rates ranged between 56 and 72% (wt.) depending on the amino acid used.

Infrared spectroscopy was used to characterise the co-monomers. The experiments were carried out on the powder samples by ATR using a Thermo Nicolet FTIR spectrometer. Absorption bands characteristic of the COOH terminal group of the co-monomers were observed around 900–1000 cm^{-1}. The presence of the C=C was confirmed by the presence of absorption bands at 900, 1200 and 1600 cm^{-1}. The co-monomers were used as obtained for the synthesis of the co-polymers.

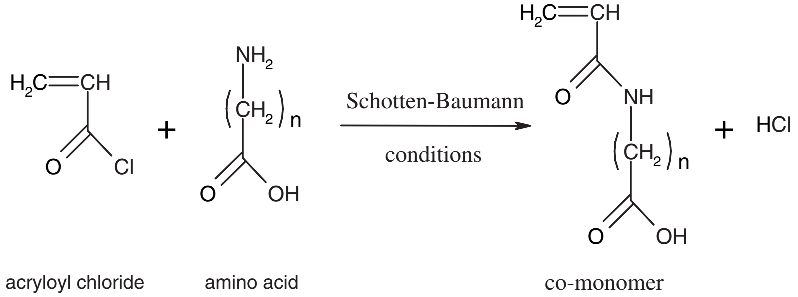


Figure 2.
Scheme of the route used for the synthesis of the co-monomers.

Synthesis of PNIPAAm and Co-Polymers

PNIPAAm and its co-polymers were synthesised by solution free radical polymerization as described by Alarcon et al.[9] (Figure 3). The same reaction conditions were used to synthesise all our polymers. The desired quantity of monomers and co-monomers were first dissolved in isopropanol in a Schlenk vessel. Subsequently the initiator (AIBN) was added to the solution. In order the remove the oxygen from the solution three freeze-thaw cycles were performed. The vessel was then placed in a thermostated oil-bath at 65 °C for 24 hours in order for the reaction to occur. The polymers were recovered by precipitation in diethyl ether and purified by dissolution and precipitation in deionised water at 50 °C. The solid precipitate was separated from the liquid phase by centrifugation at 6000 rpm for 15 minutes. The washing-centrifugation process was carried out three times. Finally the samples were freeze-dried for 2 days. The polymers were obtained in a white powder form.

Three sets of co-polymers were synthesised using the 3 co-monomers shown in figure 1. For each set three NIPAAm/co-monomer molar ratios were used: 6:1, 9:1 and 12:1. The nomenclature used to name the co-polymers is presented in Table 1. The exact composition of the co-polymer was measured using ^{1}H NMR. For this purpose the sample were dissolved in deuterated chloroform at 200 mg ml^{-1} and measurements were performed at 25 °C on a Bruker RDV500 spectrometer (500 MHz). To confirm the structure of samples the following peaks were used: 1–1.5 ppm ($C\underline{H}_3$, NIPAAm only), 1.8–2 ppm ($C\underline{H}$, polymer backbone), 1.5–3.5 ppm ($C\underline{H}_2$, co-monomers' side chains), 3.5–4 ppm ($N\underline{H}$, side chains), 4–4.5 ppm ($C\underline{H}$, neighboring CH_3 NIPAAm only) and 11–12 ppm ($COO\underline{H}$, co-monomers side chains and end groups). In order to calculate the real NIPAAm/co-monomer molar ratio of each co-polymer the following peaks at 1.5–3.5 ppm, 4–4.5 ppm and 1–1.5 ppm were used. As can be seen from Table 1 the measured molar ratios are in very good agreement with the feed ratios used.

Number and mass-average molecular weights (M_n and M_w) and the polydispersities (M_w/M_n) of the polymers were determined by gel permeation chromatography (GPC) and are listed in Table 1. A PLGel column (300 × 10 mm) was used with tetrahydrofuran (THF) (1 ml min^{-1}) as the mobile phase and dodecane as internal marker. The injection concentration was typically 0.2% of polymer in 0.1 ml THF. The eluents were monitored using a Gilson Model 132 detector. Polystyrene was used as calibration standard and therefore all the results are given in polystyrene equivalent molecular weight.

PNIPAAm was synthesised and characterised using the same procedures as described for the co-polymers. The weight-average molecular weight of the PNIPAAm used was 8100 Da.

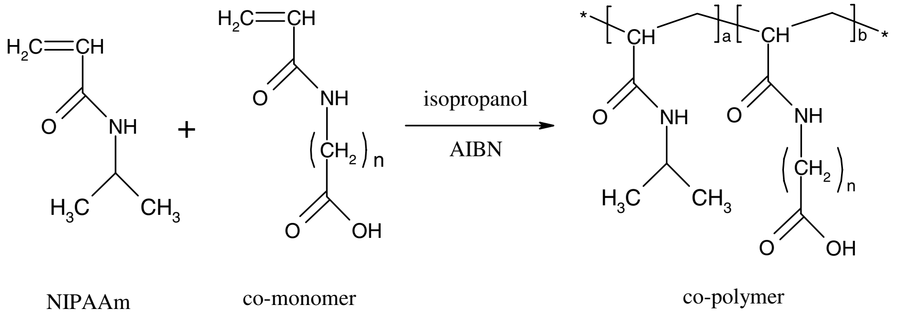
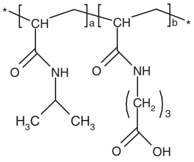
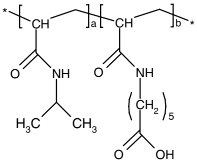
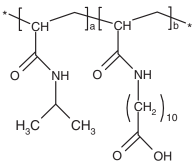


Figure 3.
Scheme of the route used to synthesise the co-polymers.

Table 1.
Structure nomenclature, composition, weight-average molecular weight and polydispersity of the copolymers synthesised.

NIPAAM/co-monomer feed molar ratio	n = 3 	n = 5 	n = 10 
Nomenclature			
6:1	—	CP(n = 5)-6-COOH	CP(n = 10)-6-COOH
9:1	CP(n = 3)-9-COOH	CP(n = 5)-9-COOH	CP(n = 10)-9-COOH
12:1	CP(n = 3)-12-COOH	CP(n = 5)-12-COOH	CP(n = 10)-12-COOH
NIPAAm/co-monomer molar composition			
6:1	—	5.6:1	6.2:1
9:1	8.9:1	9.2:1	9.1:1
12:1	11.6:1	11.3:1	11.5:1
Molecular weight (M_w)/Da			
6:1	—	2400	7900
9:1	6200	5700	6500
12:1	3800	3500	10100
Polydispersity (M_w/M_n)			
6:1	—	1.8	1.4
9:1	2.2	1.4	2.4
12:1	1.2	1.3	1.7

Differential Scanning Calorimetry (DSC)

DSC experiments were performed using a DSC Q100 from TA Instruments equipped with an autosampler and a RCS cooling system. 2.5 to 3 mg of powder samples were placed in a standard aluminum pan that was not hermetically sealed. The samples were subjected to 3 heating-cooling cycles from 10 to 200 °C at 10 °C min^{-1} under dry nitrogen atmosphere. The glass transition were measured during the 2nd and 3rd heating scan.

UV/Vis Spectrometry

UV/Vis spectrometric measurements were performed using a Perkin-Elmer Lambda 40 spectrometer at a fixed wavelength of 600 nm. Solutions were prepared in deionised water at 3 different concentrations: 1, 5 and 10 mg ml^{-1}.

Microcalorimetry

Microcalorimetric measurements were performed using a Setaram DSC III microcalorimeter. Solutions were prepared using deionised water at 20 mg ml^{-1} concentration. Standard stainless steel pans were used and deionised water was used as reference. For each sample 4 heating-cooling cycles from 5 to 60 °C at 1 °C min^{-1} were performed. The LCST temperature was recorded as the maximum of the transition peak.

Results and Discussion

In Figure 4 the T_g versus the co-monomer chain length is presented for each NIPAAm co-polymer. The glass transition for pure PNIPAAm was found to be ~130 °C.[12]

As can be seen from Figure 4 for n = 3 and n = 5 the polymer glass transition is weakly affected by the presence of co-monomers. For n = 5 and a NIPAAm/co-monomer ratio of 6:1 the glass transition is found to decrease by only 4 °C. When the side chain length is increased to n = 10 the polymer glass transition is found to decrease significantly by 20 to 30 °C

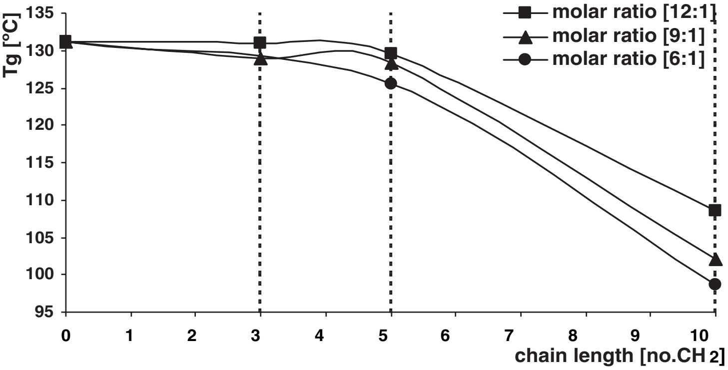


Figure 4.
Co-polymers T_g vs. co-monomer chain length for different NIPAAm/co-monomer molar ratios.

depending on the NIPAAm:co-monomer molar ratio. For all chain lengths investigated the glass transition is found to decrease with increasing co-monomer content. It is well known that the glass transition of polymers is affected by the presence of flexible side chains along their backbone.[10,11] These side chains increase the overall polymer chain mobility resulting in a decrease of the glass transition. For the set of polymers investigated here it is interesting to note that the effect of the presence of co- monomer with flexible side chains on the polymer glass transition only becomes significant for the co-monomer with the longest side chain i.e.: $n = 10$. The effect of the co-monomer on the polymer glass transition is also more pronounced for higher co-monomer content.

PNIPAAm and their co-polymers are known to have a LCST transition around body temperature. Below the LCST the polymer is soluble and clear transparent solutions are formed. When the temperature is increased above the LCST the polymer precipitates out of solution resulting in the solution becoming cloudy. The polymer through the LCST goes from an extended to a globular conformation. This transition is driven mainly by hydrophobic and

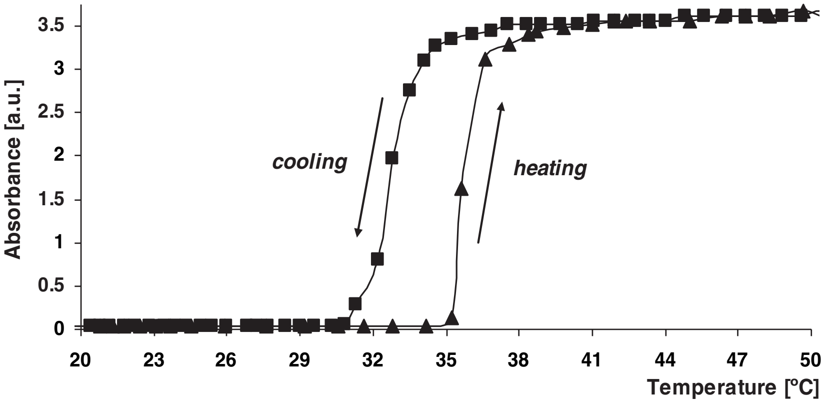


Figure 5.
UV/Vis absorbance spectra (600 nm) of a PNIPAAm solution (10 mg ml^{-1}) during a heating/cooling cycle (1 °C min^{-1}) from room temperature to 50 °C.

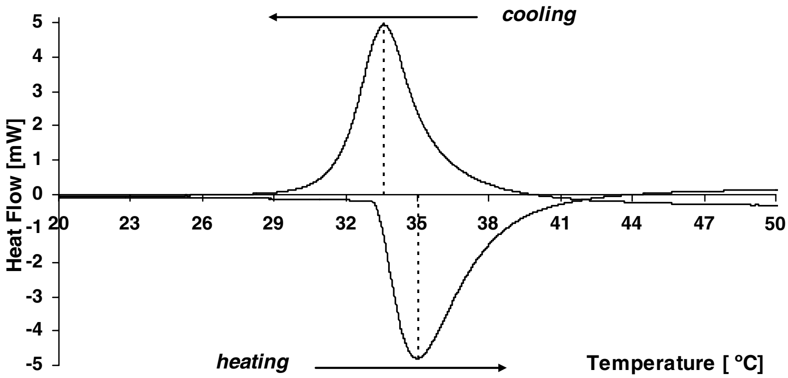


Figure 6.
Microcalorimetry curve of a PNIPAAm solution (10 mg ml^{-1}) during a heating/cooling cycle (1 °C min^{-1}) from room temperature to 50 °C.

hydrogen-bonding interactions between polymer chains. Above the LCST inter- and intra- polymer interactions become predominant resulting in the polymer falling out of solution. The transition can be followed using a UV/Vis spectrometer as shown in Figure 5 for a PNIPAAm solution at 10 mg ml^{-1}. At the LCST a step change in the absorbance of the solution is observed.

As can be seen from Figure 5 the LCST transition if fully reversible. On heating the LCST transition is usually observed at a slightly higher temperature (2 to 4 °C higher) than on cooling.

Figure 6 shows the microcalorimetry curves obtained for the same PNIPAAm solution when heated from room temperature to 50 °C and then cooled. A clear endotherm on heating and an exotherm on cooling can be observed. This suggests that at least part of the LCST process is a 1st order transition.

UV/Vis and microcalorimetry were used to measure the LCST temperatures for 20 mg ml^{-1} solutions of all our polymers. The results are summarised in Table 2.

The two techniques give identical values for the LCST temperatures. The LCST of PNIPAAm is found to be around 37 °C as expected. In Figure 7 and 8 the LCST temperatures are plotted as a function of the co-monomer content and the co-monomer chain length respectively.

As can be seen from Figure 8 for identical co-monomer content the LCST temperature decreases with increasing co-monomer chain length. As said in the introduction the longer the methylene spacer the more hydrophobic the co-monomer. Increasing the hydrophobicity of the co-monomer used results in an increase in the overall polymer hydrophobicity. Figure 8 clearly shows that the LCST is a function of the hydrophobicity of the polymer. Another effect that could be at the origin of the decrease in the LCST is the presence of acidic end groups on the co-monomers that can hydrogen bond with

Table 2.
LCSTs in heating/cooling.

	LCST temperatures (heating/cooling)/°C			
Molar ratio	n = 3	n = 5	n = 10	PNIPAAm
12:1	34.9/33.3	30.1/27.5	19.0/17.8	38.9/36.6
9:1	34.5/33.2	28.6/26.0	16.5/16.8	
6:1	—	27.8/23.0	12.3/11.5	

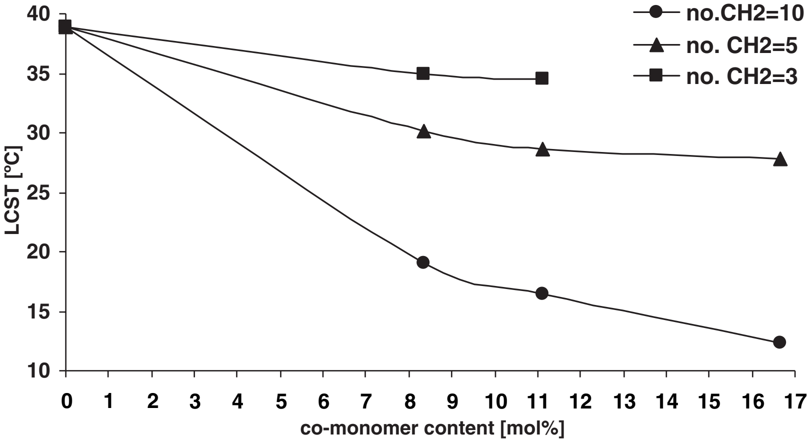


Figure 7.
LCST temperature vs. co-monomer content.

the NH groups on the PNIPAAm. This increases the probability of inter- and intra-polymer interactions stabilising the coil conformation.

For n = 3 and n = 5 in the NIPAAM/co-monomer molar ratio range the LCST seem very little affected by the increase in co-monomer content (Figure 7 and 8). On the other hand for n = 10 the doubling of co-monomer results in a significant decrease of the LCST by 10 °C.

Conclusions

The synthesis of acrylic acid derivate co-monomers with aliphatic spacers was successfully achieved with good yields (60–80%). Temperature sensitive co-polymers were prepared by the copolymerization of N- isopropylacrylamide (NIPAAm) with varying amounts of acrylamide derivates bearing carboxylic groups attached to spacers with different chain length. We have

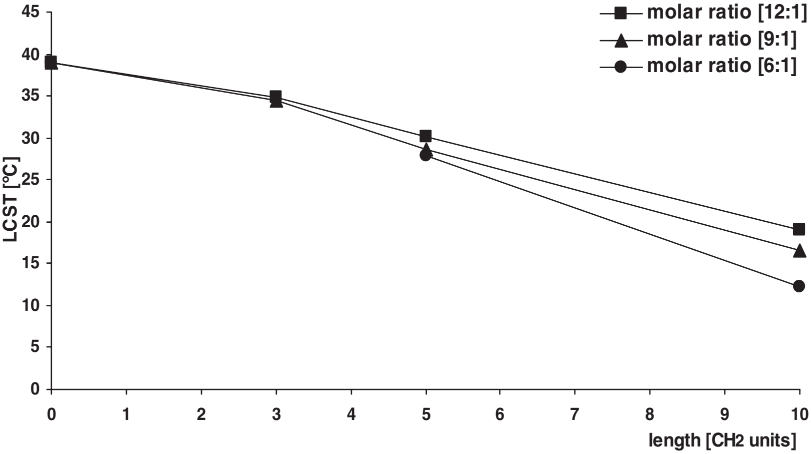


Figure 8.
LCST temperature vs. co-monomer chain length.

shown that the glass transitions as well as the LCST transition in solutions are affected by the presence of the co-monomer. The glass transition was shown to be reduced significantly for n = 10 only, while the LCST decreased continuously with increasing co- monomer chain length.

Our results clearly indicate the role played by the polymer hydrophobicity on the LCST transition. The main factor affecting the glass transition and the LCST of the polymer was found to be the side chain length of the co-monomers. In the NIPAAm/co-monomer molar ration investigated the co-monomer content was found to have an significant effect on the glass transition and the LCST only for the co-monomer with the longest methylene spacer i.e.: n = 10.

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[1] T. Okano, A. Kikuchi, Y. Sakurai, Y. Takei, N. Ogata, *J. Control Rel.* **1995**, *36*, 125.
[2] H. Schild, D. Tirrell, *Polym. Prepr.* **1989**, *30*, 342.
[3] G. Chen, A. S. Hoffamn, *Macromol. Chem. Phys.* **1995**, *196*, 1251.
[4] E. Ayano, H. Kanazawa, *J. Sep. Sci.* **2006**, *29*, 738– 749.
[5] T. F. D. J. Tanaka, T. Sun, I. Nishio, G. Swislow, A. Shah, *Phys. Rev. Lett.* **1980**, *45*, 1636.
[6] C. d. l. H. Alarcon, S. Pennadam, C. Alexander, *Chem. Soc. Rev.* **2005**, *34*, 276–285.
[7] D. Kuckling, H. J. P. Adler, K. F. Arndt, L. Ling, W. D. Habicher, *Macromol. Chem. Phys.* **2000**, *201*, 273.
[8] D. E. Frantz, D. G. Weaver, J. P. Carey, M. H. Kress, U. H. Dolling, *Org. Lett.* **2002**, *4*, 4717–4718.
[9] C. d. l. H. Alarcon, Vol. PhD, University of Portsmouth, **2004**.
[10] S. Kasapis, *Food Hydrocoll.* **2006**, *20*, 218–228.
[11] G. Dlubek, D. Bamford, A. Rodriguez-Gonzales, S. Bornemann, J. Stejny, B. Schade, M. A. Alam, M. Arnold, *J. Polym. Sci. : Part B: Polymer Physics* **2002**, *40*, 434–453.
[12] J. Brandrup, E. H. Immergut, *Polymer Handbook* **1989**.
[13] G. H. Schild, *Prog. Polym. Sci.* **1992**, *17*, 163.