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Degradable thermoresponsive polymers which display redox-responsive LCST Behaviour†

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Disulfide linkages were introduced into poly (*N*-isopropylacrylamide) by the polycondensation of a RAFT-derived, telechelic macromonomer to give degradable yet vinyl-based polymers. These polymers displayed a redox-sensitive lower critical solution temperature (LCST) with the shorter, degraded product displaying a higher LCST than its non-degraded counterpart.

Macromolecules which display lower critical solution temperature (LCST) behaviour have attracted significant attention as 'smart' materials.[1] Heating an aqueous polymer solution above its LCST initiates an entropically favourable coil-to-globule transition. This is driven by the expulsion of water molecules associated with the polymer chain and the formation of interchain hydrogen bonds. This reversible (or 'smart') behaviour has been exploited, for example, to reversibly precipitate enzymes,[2] to modulate protein catalytic activity[3] and to functionalise nanoparticles.[4–5] Recently the LCST switch, which can be considered to modulate polymer hydrophobicity/lipophilicity, has been investigated as a route to selectively trigger cellular uptake[6–8] and has been demonstrated by the enhanced uptake of poly (*N*-isopropylacrylamide), (pNIPAM), in mice with hyperthermic tumour tissues.[9] If *in vivo* requirements are to be considered, a macromolecular carrier should have a molecular weight above the renal filtration limit of approximately 40 000 g mol^{-1}.[10] However, the toxicity associated with the indefinite accumulation of foreign bodies in tissues necessitates the inclusion of a degradation mechanism within the polymer backbone. Polyesters and polyamides can be degraded by hydrolytic and/or enzymatic processes. However, these processes are non-specific with the resulting complex mixture of by-products potentially compromising upon performance.[11] Furthermore, the drop in pH resulting from carboxylic acid generation can also cause inflammation.[12] The degradation of poly (propylene sulfide), *via* a non-specific, oxidative pathway,[13] and self-immolating polymers, *via* a series of cascade reactions, has also been demonstrated.[14] An alternative strategy is to incorporate reduction-sensitive disulfide units into the material structure. This is of particular relevance given the presence of glutathione, a powerful reducing agent, *in vivo*. Furthermore, given glutathione is present at micromolar levels in the systemic circulation, but several thousand times higher (millimolar) inside cells, this concentration gradient can be exploited to trigger degradation following cellular internalisation, and has been studied in the field of gene therapy.[6,15] Poly-esters, -amides and -disulfides are generally accessed by polycondensation or ring opening polymerisation methods. The low functional group tolerance inherent to these techniques does however limit the range of possible materials.[15–17] Conversely, controlled radical polymerisation techniques such as reversible addition-fragmentation chain transfer (RAFT) polymerisation not only allow the preparation of materials with exquisite control over molecular weight and polydispersity, but also enable the incorporation of more advanced functionality.[18] A key challenge therefore is the use of such methodologies to synthesise biodegradable polymers.

In this contribution, we introduce a new method to generate reduction-sensitive, degradable polymers from RAFT derived macromonomers containing a thermoresponsive component. The polymer is tailored such that following degradation, the LCST increases dramatically enabling the "thermosensitivity" to be "switched off", thereby promoting by-product re-solubilisation. This strategy may also avoid any cytotoxicity associated with insoluble (above LCST) polymers inside cells/tissue.

The thermoresponsive polymer selected in this study was pNIPAM[19] given its biocompatibility and known molecular weight-dependent LCST behaviour (*vide infra*).[20] Well-defined pNIPAMs (M_n = 1700, 4400 and 9800 g mol^{-1}) were synthesised by RAFT polymerisation (see ESI†) and their cloud points measured as a function of concentration (Fig. 1). Polymers with M_n = 4400 and 9800 g mol^{-1} both had cloud points around 32 °C in the range 1–5 mg mL^{-1}. Interestingly, when M_n = 1700 g mol^{-1} the cloud point was observed to increase from 37 to 55 °C as the concentration was decreased from 5 to 1 mg mL^{-1}. Considering this data, it was decided in order to obtain the largest possible LCST "switch" following degradation (*vide infra*), the molecular weight should shift from above 10 000 g mol^{-1} to below 2000 g mol^{-1}.

The proposed synthesis of a bioreducible, disulfide-containing pNIPAM from a macromonomer bearing dithioester and pyridyl disulfide groups at the ω- and α-termini respectively is illustrated in Fig. 2A. This was achieved by exploiting the inherent end-group control offered by RAFT,[21] as described

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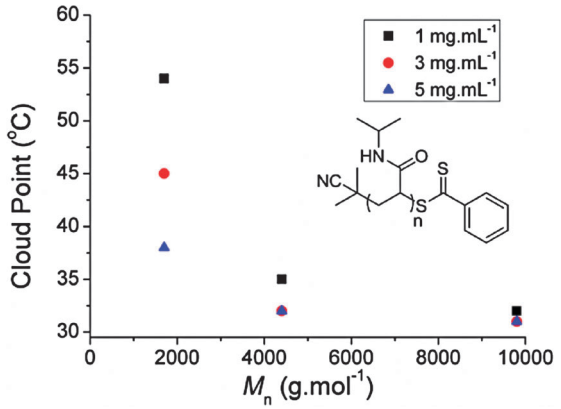


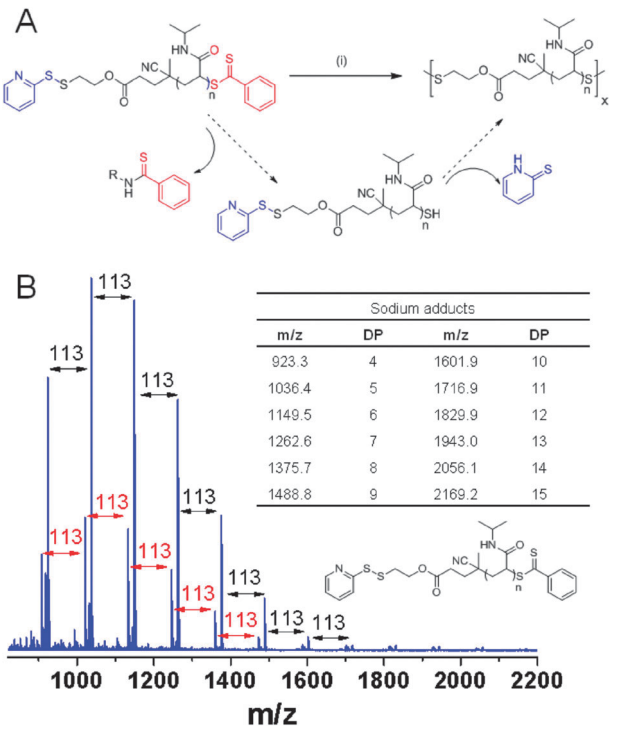
Fig. 1 Cloud points of pNIPAM as a function of concentration and molecular weight. Chemical structure of pNIPAM is inset.

by Maynard *et al.*[22] A new RAFT agent, 2-(pyridyldisulfanyl)-ethyl 4-cyano-4-(phenylcarbonothioylthio)-pentanoate, PECPP, was used to install these functionalities. PECPP was synthesised by reaction of hydroxyethyl pyridyl disulfide with 4-cyano-4-(phenylcarbonothioylthio)pentanoic acid using *N*,*N*′-diisopropylcarbodiimde as the coupling reagent (for synthetic and characterisation details, see ESI†). Selective cleavage of the dithioester to give a ω-thiol group can then allow for a polycondensation-type reaction of the macromonomer, driven by the expulsion of pyridine thione.[22]

NIPAM was polymerised in toluene/methanol (1 : 1) at 70 °C using PECPP as chain transfer agent. SEC analysis of the purified polymer gave M_n = 1300 g mol^{-1} and M_w/M_n = 1.35. Given the presence of base in the SEC eluent, the formation of terminal thiols was a possibility. Analysis was therefore performed in the presence of tributyl phosphine to reduce both end groups and prevent any potential oxidative- or thiol-pyridyl disulfide- induced coupling (see ESI for SEC traces†). ^{1}H NMR analysis qualitatively indicated the desired dithioester and pyridyl disulfide end-groups had been installed whilst MALDI-ToF quantified the presence of both end groups on every chain. This is shown in Fig. 2B where each peak corresponds to n-NIPAM repeat units, the mass of both end groups and a lithium/sodium ion. The molecular weights agree well with the values obtained by SEC ruling out mass discrimination.

This well-defined macromonomer was subsequently polymerised to a disulfide-linked polymer *via* a polycondensation-type reaction. 1 equivalent of ethanolamine and 2 equivalents of triethylamine (TEA) were used to cleave the dithioester at the ω-terminus. The TEA was essential and is hypothesised to increase the nucleophilicity of the terminal thiol through deprotonation. To prevent pyridyl disulfide cleavage, the addition of excess ethanolamine was avoided. Oxygen was rigorously removed by a minimum of 3 freeze-pump-thaw cycles to prevent oxidative coupling of terminal thiol groups. Following 24 h of reaction at 25 °C, SEC analysis revealed a significant increase in M_w from 1750 to 34 000 g mol^{-1} (Fig. 3). Given the observed molecular weight was considerably higher than that expected from oxidative, thiol–thiol coupling reactions (*i.e.* dimer formation), the polymerisation was deemed successful. Thiol-pyridyl disulfide coupling was confirmed as the predominant mechanism in this case since control experiments, performed in the presence of oxygen, produced a peak with M_n = 2600 g mol^{-1}, equivalent to oxidative thiol–thiol coupling.

Quantitative removal of the RAFT agent was demonstrated by use of SEC-coupled photodiode array (see ESI†) and near-identical molecular weights were obtained when the reaction was performed at several concentrations (40, 20, 10, 1 mg mL^{-1}). The presence of disulfides in the backbone was confirmed by adding the reducing agent tributyl phosphine to the polymer solution, which resulted in a decrease in molecular weight and a



Sodium adducts

m/z	DP	m/z	DP
923.3	4	1601.9	10
1036.4	5	1716.9	11
1149.5	6	1829.9	12
1262.6	7	1943.0	13
1375.7	8	2056.1	14
1488.8	9	2169.2	15

Fig. 2 (A) Polycondensation using pNIPAM macromonomer: (i) Ethanolamine (1 molar equiv.); triethylamine (2 molar equiv.); THF; 25 °C; 24 h). (B) MALDI-ToF spectrum of telechelic polymer (inset). Table shows peak assignments for sodium adducts (indicated in black). Series of peaks corresponding to lithium adducts (red) are also shown, and peaks listed in supporting information.†

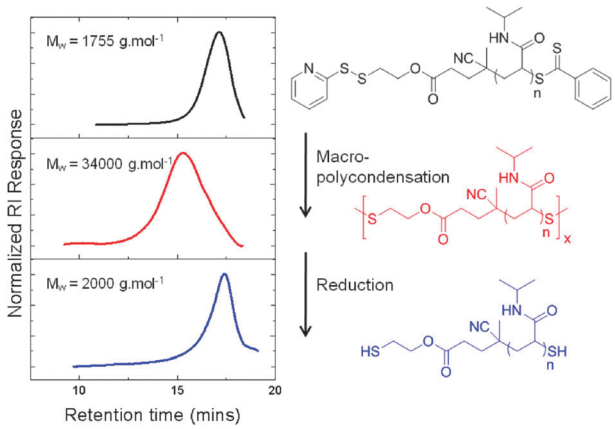


Fig. 3 SEC analysis demonstrating successful polymer "condensation" procedure using a telechelic macromonomer. Reduction was achieved using tributyl phosphine.

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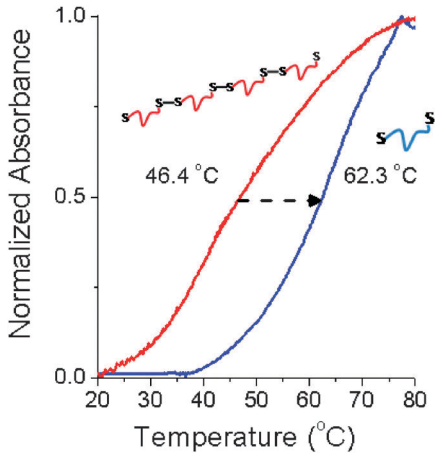

Fig. 4 Turbidimetry curves showing shift in LCST of pNIPAM following reduction of backbone disulfide bonds. Red trace is disulfide linked polymer (M_w = 34 000 g mol^{-1}) and blue trace is reduced polymer (M_w = 1750 g mol^{-1}).

distribution identical to that of the starting material. These experiments demonstrate that RAFT-derived telechelic macromonomers can be used to obtain high molecular weight polymeric carriers which specifically degrade in the presence of reducing agents to well-defined oligomeric by-products.

The primary goal of this work was to use disulfide linkages as (i) a new route towards selective degradation and (ii) as a secondary stimulus to shift the LCST of the polymer and produce soluble by-products. The cloud point of the disulfide-containing-polymer product was measured before and after reduction by TCEP (tris-2-carboxyethylphosphine), Fig. 4. Considering the strong concentration-dependence of this property, these experiments were conducted at 0.05 mg mL^{-1}, close to what might be expected in a biological application.[23] The cloud point shifted from 46 °C to 62 °C following addition of TCEP and can be directly correlated to the decrease in molecular weight. Both high and low molecular weight pNIPAMs were indicated to be biocompatible with red blood cells both above and below the cloud point up to 3 mg mL^{-1} (see ESI†).

In conclusion, this manuscript has demonstrated a powerful new method to incorporate degradable linkages into the backbone of RAFT-derived poly (*N*-isopropylacrylamide), with the potential for extension to other functional monomers. The degradation of the polymer promoted a secondary response, namely an increased LCST following addition of a suitable reducing agent. This provided an "off switch" allowing the hydrophilic/hydrophobic transition to be reversed without the need to lower the solution temperature. This method may find application for triggered cellular uptake, where intracellular glutathione can degrade the polymer, releasing any cargo before allowing re-solubilisation of the polymer by-products. Future studies will focus on (i) fine-tuning the transition temperature; (ii) stimuli-responsive cellular uptake *in vitro*; (iii) incorporation of drugs into the polymer side chains *via* cleavable units.

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