Cleavage of Macromolecular RAFT Chain Transfer Agents by Sodium Azide During Characterization by Aqueous GPC

Reece W. Lewis[a], Richard A. Evans[b], Nino Malic[b], Kei Saito[c]*, Neil R. Cameron[a,d]*

[a]Department of Materials Science and Engineering, Monash University, 22 Alliance Lane, Clayton, Victoria, 3800, Australia

[b]CSIRO Manufacturing Flagship, Clayton, 3168, Australia

[c]School of Chemistry, Monash University, Clayton, 3800, Australia

[d]School of Engineering, University of Warwick, Coventry, CV4 7AL, UK

Abstract

Accurate and reliable analysis of polymers by GPC is vital in the field of controlled radical polymerisation. Often, water-soluble polymers are analysed by aqueous gel permeation chromatography (GPC) in a solvent containing dilute sodium azide as an anti-microbial agent. Previous reports have shown that sodium azide at high concentration is able to remove terminal CTA groups from polymer chains, producing thiol-terminated polymers. This study demonstrates that GPC sample preparation of RAFT polymers in aqueous solvents containing dilute (200 ppm) sodium azide can cause significant changes in the measured molecular weight distribution. These changes occur within hours of dissolving the polymer sample and are shown to be due to cleavage of the CTA in the polymer chain together with disulfide coupling of the resulting polymeric thiols. The extent to which this occurs is strongly dependent on the CTA attached to the polymer; an almost 10-fold difference in the rate of CTA removal is observed between different RAFT agents. The by-product of the reaction between sodium azide and RAFT polymers is also investigated and shown to be an unstable thiatriazole-functionalised Z group. The thiatriazole then degrades further to form a nitrile-functionalised Z group, N_2 and elemental sulfur.

Introduction

Reversible addition-fragmentation chain transfer (RAFT) polymerisation is a widely used reversible deactivation radical polymerisation (RDRP) technique for the generation of low dispersity, controlled polymers.[1] With the addition of a compatible chain transfer agent (CTA) to a free radical polymerisation, the facile synthesis of controlled architectures such as block, star and graft copolymers can be achieved.[2, 3] Due to its compatibility with a wide range of monomers and experimental conditions (including aqueous solvents), RAFT has proven to be a particularly versatile and popular technique.[4]

RAFT polymerisations have been widely conducted in aqueous solvents in cases where compatibility with organic solvents is poor. For example, bioconjugation of RAFT polymers to proteins is conducted in aqueous solution both for solubility reasons and to prevent protein denaturation. Another reason for the use of aqueous solution is poor monomer/polymer solubility in organic solvents, as can be the case for hydrophilic and charged monomers. Furthermore, associated environmental and economic benefits can also be a driver for performing RAFT reactions in aqueous solution.[5, 6] Further benefits can arise from conducting polymerisations at room temperature; this has been demonstrated for the synthesis of multiblock copolymers and ultra-high molecular weight copolymers initiated by either ϒ-irradiation or redox methods.[7-9] Recently, Sumerlin and coworkers demonstrated the preparation of ultra-high molecular weight poly(*N*,*N*-dimethylacrylamide) by photoinitiated RAFT polymerisation in aqueous solution at mild temperature.[10]

Gel permeation chromatography (GPC) is the technique to characterise and analyse the average molecular weight and the molecular weight dispersity of synthesised polymers. For water-soluble polymers such as polyacrylamide, aqueous GPC is often used. We have found that a shoulder at approximately half the peak molecular weight of the sample could be observed when we analysed synthesised, under aqueous conditions, high molecular weight acrylamide polymers with a symmetrical trithiocarbonate CTA. The solvent used to prepare samples for GPC was an aqueous pH 8 buffer with 200 ppm sodium azide. Sodium azide is often added to aqueous GPC systems as a biocide and has been used by various research groups for analysing RAFT polymer samples (at concentrations from 75 to 300 ppm).[7, 9, 11-13]

In previous research, sodium azide has been shown to be an effective nucleophile for removing terminal dithiobenzoate CTA groups from the ends of polymer chains.[14] Analogous to the reaction with primary amines, they demonstrated that a thiol-terminated polymer is formed which can then undergo oxygen-facilitated disulfide coupling. Thus, they were able to monitor the reaction by GPC through the emergence of a second peak at double the molecular weight of the original sample. Using 267 mM sodium azide a bimodal distribution was often observed within minutes. In our case, the trithiocarbonate CTA group is located in the middle of the polymer chain, therefore the expected outcome would be the emergence of a new peak at half the starting molecular weight. This predicted outcome is consistent with our aqueous GPC results and encouraged us to perform further investigation.

In this manuscript, we have studied the effect of sodium azide in aqueous GPC solvent on the analysis of RAFT polymers. At the concentration of sodium azide used in our aqueous

GPC solvent (200 ppm), an alteration to MWD was observed during GPC sample preparation. We then compared the rate of azide attack on a range of polymeric CTAs, both at typical GPC solvent concentrations and at higher concentrations. Significant differences in rate of reaction with azide were found, depending on CTA molecular structure. Additionally, the reaction mechanism and nature of the side products formed was examined.

Experimental

Materials

N,*N*-dimethylacrylamide (DMA, 99%) was obtained from Sigma Aldrich and passed through basic alumina prior to use to remove inhibitor. 1,1'Azobis(cyclohexanecarbonitrile) (Vazo-88, 98%) and azobisisobutyronitrile (AIBN, 98%) were obtained from Sigma Aldrich and purified by recrystallising from methanol. Acrylamide (AM, 98%), sodium formaldehydesulfoxylate (NaFS), ammonium persulfate (APS, 98%), NaN_3 (99.5%), Na_3PO_4 (96%) and methylamine (98%) were obtained from Sigma Aldrich and used as received. 4,4'-Azobis(4-cyanopentanoic acid) (VA-501, 98% MP Biomedicals), $NaNO_3$ (99.5% VWR Chemicals), methyl ethyl ketone (MEK, VWR Chemicals), acetonitrile (Ajax Chemicals), diethyl ether (RCI Labscan), acetone (Chem-Supply), ethanol (Chem-Supply) and petroleum spirit (Merk) were obtained from various suppliers and used as received.

The chain transfer agents 2-(((butylthio)carbonothioyl)thio)propanoic acid (SPAT)[15] and S,S'-bis(α,α'-dimethyl-α''-acetic acid)trithiocarbonate (DMAT)[16] were synthesised according to literature procedures, while O-ethyl-S-(1-methoxycarbonyl) ethyl dithiocarbonate (XA01)[17] was synthesised by the reaction of potassium ethyl xanthogenate with methyl 2-bromo-propionate (modified literature procedure). Analytical data were in accordance with

literature values. 3-((((1-carboxyethyl)thio)carbonothioyl)-thio)propanoic acid (BM1429, 90%), cyanomethyl (3,5-dimethyl-1*H*-pyrazole)-carbodithioate (CDMPC, 95%) and dibenzyltrithiocarbonate (DBTTC, 97%) were obtained from Boron Molecular and used as received. 2-Cyano-2-propyl benzodithioate (CPDB, 97%) was obtained from Sigma Aldrich and used as received.

Deuterium oxide (D_2O, 99.9% D atom) and deuterated chloroform ($CDCl_3$, 99.8% D atom) were obtained from Cambridge Isotope Laboratories and used for ^{1}H NMR and ^{13}C NMR analysis.

Methods

NMR spectroscopy

^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz) using either D_2O or $CDCl_3$.

Gel permeation chromatography (GPC)

The average molecular weight and dispersity (Đ) of the resultant polymers was measured through gel permeation chromatography (GPC). Samples were analysed on either a Shimadzu DMAc system (5.3kCPDB polymers) or a Waters Alliance system (all other samples). The Shimadzu system is equipped with a CMB-20A controller system, an SIL-20A HT autosampler, an LC-20AT tandem pump system, a DGU-20A degasser unit, a CTO-20AC column oven, an RDI-10A refractive index detector, and 4× Waters Styragel columns (HT2, HT3, HT4, and HT5, each 300 mm × 7.8 mm^2, providing an effective molar mass range of 100 to 4 × 10^6). *N,N*-Dimethylacetamide (DMAc) (containing 4.34 g L^{-1} lithium bromide (LiBr))

was used as an eluent with a flow rate of 1 mL/min at 80 °C. Number (M_n) and weight average (M_w) molar masses were evaluated using Shimadzu LC Solution software. The GPC columns were calibrated with low dispersity poly(methyl methacrylate) (PMMA) standards (Polymer Laboratories) ranging from 1.01 x 10^3 to 2.13 x 10^6 g mol^{-1}, and molar masses are reported as PMMA equivalents. The Waters Alliance system is equipped with an Alliance 2695 Separations Module (integrated quaternary solvent delivery, solvent degasser and autosampler system), a Waters column heater module, a Waters 2414 RDI refractive index detector and 2× Agilent PL-AquaGel-OH columns (Mixed H, 8µm), each 300 mm × 7.8 mm^2, providing an effective molar mass range of 100 to 10^7. Aqueous buffer was prepared containing 0.2 M $NaNO_3$, 0.01M Na_3PO_4 in Milli-Q water with 200ppm NaN_3 and adjusted to pH 8 and filtered through 0.45µm filter. The filtered aqueous buffer was used as an eluent with a flow rate of 1.0 mL/min at 30°C. The GPC columns were calibrated with low dispersity PEO standards (Polymer Laboratories) ranging from 238 to 969,000 g mol^{-1}, and molar masses are reported as PEO equivalents. A 3rd-order polynomial was used to fit the log M_p vs. time calibration curve for both systems, which was near linear across the molar mass ranges.

UV-vis spectroscopy

The progress of CTA group removal was monitored through UV-vis spectroscopy (Agilent Technologies, Cary 60, UV-vis spectrophotometer). In each measurement a 1 cm path length quartz cuvette was used with DI water as the solvent.

FTIR spectroscopy

Attenuated total reflectance (ATR) FTIR spectroscopy was performed on a Thermo Scientific Nicolet 6700 FTIR spectrometer using a diamond crystal. Data are collected between 4000 and 525 cm^{-1} by summing 32 scans with a resolution of 4 cm^{-1}.

Polymer Synthesis by RAFT polymerisation

Nine different polymer samples were prepared by RAFT polymerisation employing a range of CTAs (**Figure 1**). The key experimental parameters for each polymer sample are given **Table 1**. Polymers **378kDMAT** and **365kDMAT** were prepared by a Chemspeed automated parallel synthesiser; all other polymers were prepared using standard round-bottom flask procedures. Full experimental details are provided in the accompanying Supporting Information file.

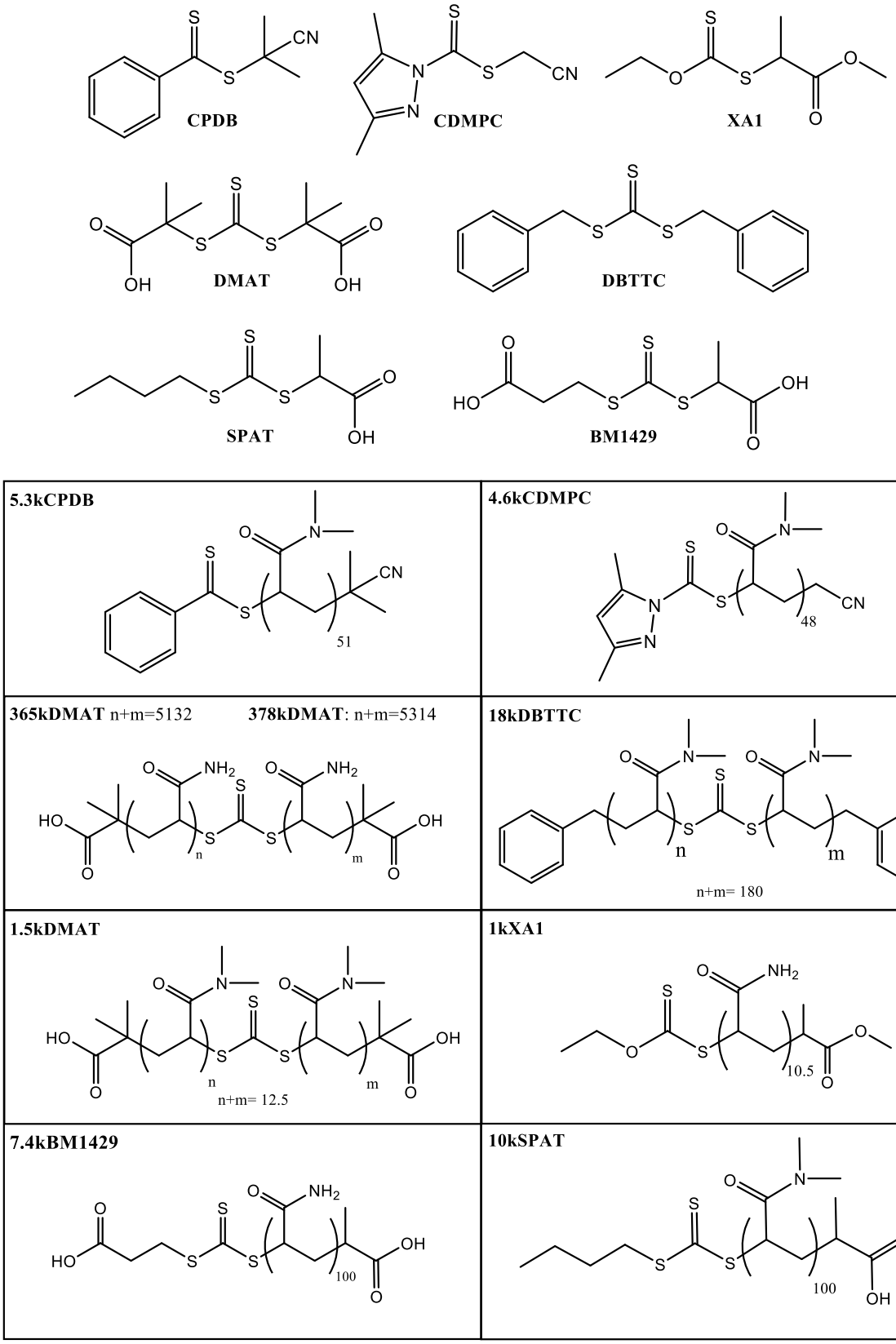

Figure 1. Molecular structure of CTAs and synthesised RAFT polymers. For sample naming scheme, see footnote to Table 1.

Table 1. Composition and Properties of Synthesised RAFT Polymers.

Sample name[a]	**CTA**	**Monomer**	**$M_{n,conv}$ (^{1}H-NMR)**[b]	**CTA λ_{max}**[c]
5.3kCPDB	CPDB (dithiobenzoate)	Dimethylacrylamide	5.3 kDa	305 nm
4.6kCDMPC	CDMPC (dithiocarbamate)	Dimethylacrylamide	4.6 kDa	303 nm
365kDMAT	DMAT (trithiocarbonate)	Acrylamide	365 kDa	305 nm
378kDMAT	DMAT (trithiocarbonate)	Acrylamide	378 kDa	305 nm
18kDBTTC	DBTTC (trithiocarbonate)	Dimethylacrylamide	18 kDa	305 nm
1.5kDMAT	DMAT (trithiocarbonate)	Dimethylacrylamide	1.5 kDa	306 nm
1kXA1	XA1 (xanthate)	Acrylamide	1.0 kDa	279 nm
7.4kBM1429	BM1429 (trithiocarbonate)	Acrylamide	7.4 kDa	302 nm
10kSPAT	SPAT (trithiocarbonate)	Dimethylacrylamide	10 kDa	309 nm

[a]Samples named according to XkY where X=$M_{n,conv}$ (kDa), Y=CTA abbreviation.

[b]$M_{n,conv} = Mw_{monomer} \times \frac{[Monomer]_0}{[CTA]_0} \times \%Conversion + Mw_{CTA}$ where %conversion is calculated by comparing the ^{1}H-NMR integrals of the unsaturated double bond on the monomer to the polymer backbone.

[c]CTA λ_{max} is λ_{max} for $260 < \lambda < 350$ nm and was used to track the respective CTA concentration for UV-vis spectroscopy studies.

Cleavage of Macro-CTAs

Initial experiments investigated the stability of the trithiocarbonate group under GPC solvent conditions to investigate the cause of the observed bimodal distribution after aqueous GPC characterisation. **378kDMAT** and **365kDMAT** were dissolved in the solvents listed below at a concentration of 24 mg polymer/mL. This concentration was chosen to deliver a UV-vis absorbance at λ_{max} close to 1 without further dilution to allow for easy continuous monitoring. The effect of each solvent was investigated by measuring the change in UV-vis absorbance and molecular weight distribution over time.

1. Deionised water
2. Aqueous GPC solvent (0.2 M $NaNO_3$, 0.01M Na_3PO_4, 200 ppm NaN_3, adjusted to pH 8)
3. Azide-free aqueous GPC solvent (0.2 M $NaNO_3$, 0.01M Na_3PO_4, adjusted to pH 8)
4. 200 ppm sodium azide (aqueous)

This experiment was then repeated with a different trithiocarbonate CTA polymer (**7.4kBM1429**) at a concentration of 1 mg/mL.

To further understand the effect of CTA type on the stability of polymer samples, a wider range of polymers were treated with aqueous sodium azide at concentrations representative of GPC conditions. The polymers were dissolved in an aqueous solution of 3.1 mM (200 ppm) sodium azide at a polymer concentration of 0.1 mM (2-0.1 mg/mL depending on the polymer molecular weight). Additionally, selected samples were also

analysed at a higher concentration of sodium azide with the rate of CTA removal compared to that achieved by methylamine (267mM sodium azide/methylamine and 26.7 mM polymer). The change in UV-vis absorbance (for the corresponding CTA) was then monitored over time to track the reaction progress. For UV-vis measurements, the 0.1 mM polymer samples were analysed *in situ* (without dilution), whereas the 26.7 mM polymer solutions were diluted with DI water to 0.13 mM.

Mechanistic Studies

A slightly modified experimental method to that given in the section above for CTA removal of **4.6kCDMPC** with 267mM sodium azide was carried out to produce a greater amount of polymer precipitate for analysis. In short, 600 mg of **4.6kCDMPC** was dissolved in 4.5 mL of 267 mM sodium azide solution. After 4 hours, the resultant precipitate was filtered under vacuum and washed with DI water (20mL). The washed solid was then dried in a vacuum oven at 40^{o}C for 3 hours.

Results and Discussion

Aqueous GPC Solvent Investigations

We initially investigated the extent of azide-induced CTA cleavage with time for polymer **365kDMAT** as a representative high molecular weight polyacrylamide with a trithiocarbonate CTA in the middle of the chain (**Figure 1**). Cleavage was monitored over time by UV-Vis spectrophotometry as is shown in **Figure 2**. The results clearly demonstrate a continual reduction in absorbance at 302 nm (λ_{max} for trithiocarbonates) over time for the RAFT polymer dissolved in azide-containing solvents, while either water alone or basic

buffer results in no change (note that the GPC buffer itself has an absorbance at 302 nm, hence the difference in initial absorbances in **Figure 2**). In this case, the rate of loss of absorbance appears to be approximately 50% after 45 hours in 200 ppm sodium azide. Figure 2 suggests that even at 2.5 hours it is likely that some alteration of the molecular weight distribution will have occurred due to attack on the CTA. With the time for polymer dissolving, dispersion, sample loading and column preparation taken into account for GPC analysis, 2.5 hours can often be the minimum amount of time a sample spends in a GPC solvent (particularly for high molecular weight samples where longer dissolution times are needed). Considering the low targeted dispersities of most RAFT polymerisations, it is likely even a slight alteration of MWD will be undesirable to the researcher.

In analysing RAFT polymer samples after CTA nucleophilic cleavage it is important to consider how the CTA location (terminal or central) and the presence (or lack) of an acrylic monomer in the sample will affect the expected alteration of molecular weight distribution (**Figure 3**). Nucleophilic attack on polymers with a terminal CTA group will produce thiol-terminated polymers of unchanged molecular weight which may then undergo disulfide coupling, producing chains of twice the original molecular weight (Figure 3, part 1), or alternative side reactions such as back-biting as has been observed for polymethacrylates[18]. However, if residual acrylic monomer is left in the sample, Michael addition to chain end thiols may also occur.[19, 20]

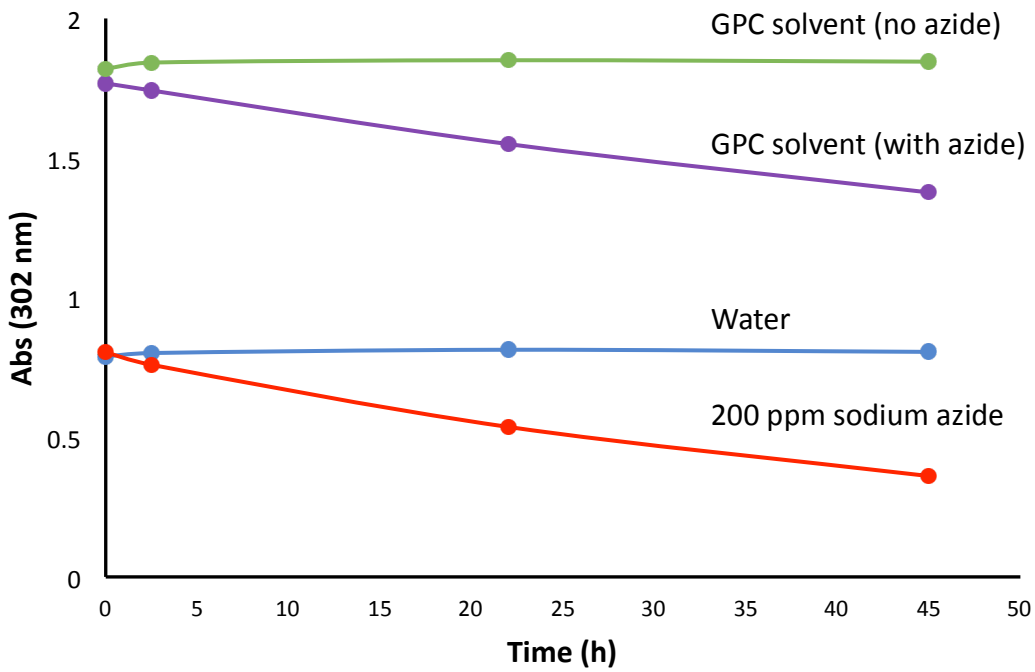

Figure 2. CTA group loss from **365kDMAT** in different solvents monitored by UV-Vis absorbance at 302nm (λ_{max} for trithiocarbonates).

In the case of trithiocarbonates, the thiocarbonyl compound formed by reaction with sodium azide may or may not be susceptible to a second nucleophilic attack. Figure 3 illustrates the outcomes of sodium azide attack on symmetrical RAFT polymers (CTA group in the middle of the chain) for both of these cases (Figure 3, parts 2 and 3). An initial nucleophilic attack will produce two polymer chains of approximately half the molecular weight of the parent chain. One of these polymer chains will be thiol-terminated and the other thiocarbonyl-terminated. Assuming that the thiocarbonyl group is inert, then even in the absence of acrylic monomer only half of the cleaved chains can re-combine; theoretically resulting in a bimodal distribution with a M_n which is overall 75% of the original value.

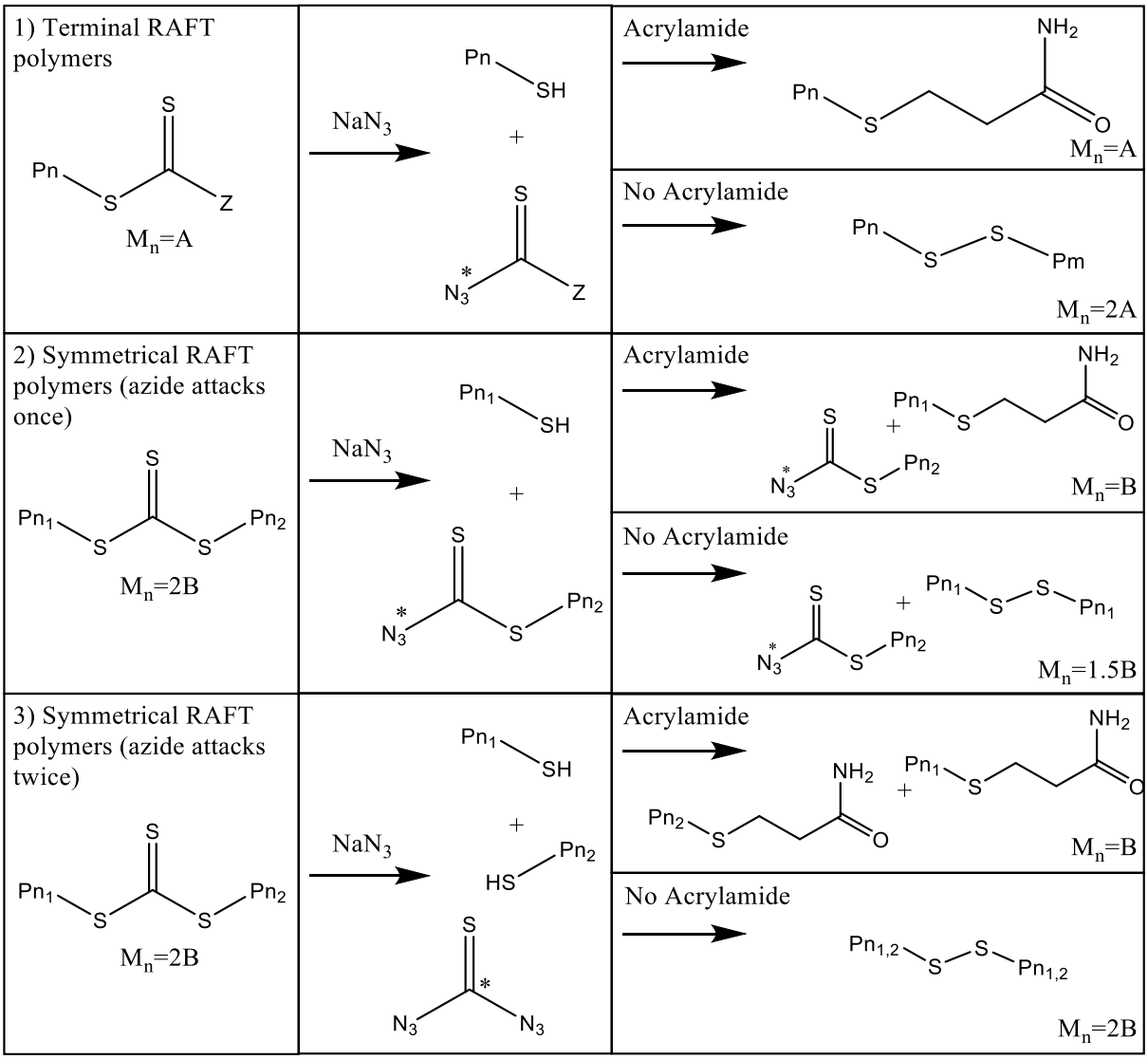

Figure 3. Possible molecular weight alterations from sodium azide attack on RAFT polymers depending on CTA type and the presence or lack of residual acrylic monomer (acrylamide shown as an example). The structures labelled * are for illustrative purposes only and may not be stable in this form.

If, however, the terminal thiocarbonylthio group is able to produce a second thiol terminated chain (by further reacting with sodium azide) then a complete recovery of molecular weight distribution (in the absence of acrylic monomer) is possible through

disulfide coupling. In either case, the prevention of disulfide coupling due to thiol reaction with residual monomer will result in a halving of M_n. Thus, removal of all residual monomer before dissolving in GPC solvent may limit the effect on symmetrical RAFT polymers.

In order to confirm that the reduction of UV absorbance at 302 nm corresponded to cleavage of the trithiocarbonate group by reaction with sodium azide, the molecular weight distribution of **365kDMAT** was measured by GPC (**Figure 4**). Firstly, it is shown that samples prepared in solvents without sodium azide produce a symmetrical distribution with $M_{n,GPC}$ in good agreement with $M_{n,conv}$ (Table S1, supporting information). Additionally, there is no difference in Đ between samples in water or the pH 8 GPC solvent without sodium azide. A clear difference arises when these are compared with the sodium azide-containing solvents, which produce a clear bimodal distribution. It can be seen that approximately half of the polymer chains have halved in molecular weight, resulting in a second peak of similar magnitude at approximately 250 kDa. The observation that approximately half of the polymer chains have halved in molecular weight is in strong agreement with the near-halving of UV-Vis absorbance at 302 nm.

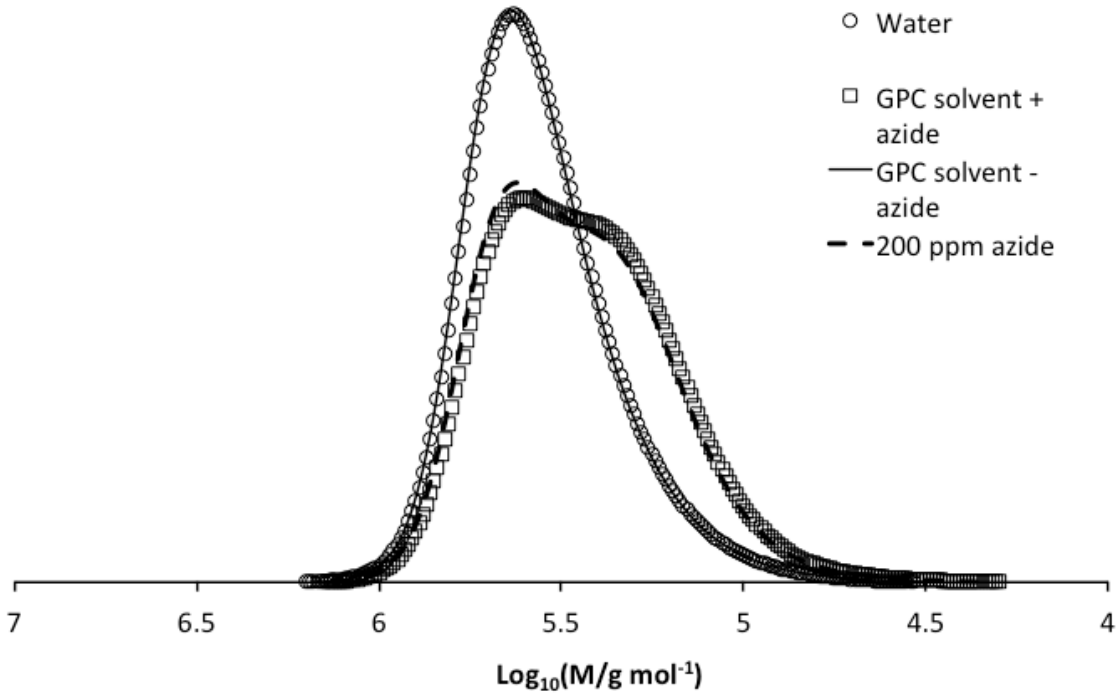

Figure 4. Molecular weight distributions as determined by GPC for **365kDMAT** after 48 hours in different solvents.

The experiment was then repeated with **7.4kBM1429** to confirm that polymers with a terminal CTA will similarly be cleaved in GPC solvents containing 200 ppm sodium azide. It was noted that this RAFT polymer experienced less than 6% reduction in absorbance at 305 nm, even after 48 hours in either the GPC solvent (with azide) or 200 ppm sodium azide (Figure S1, supporting information). The corresponding GPC trace showed an emerging peak at double the starting molecular weight, suggesting that cleavage of the CTA end group is accompanied by disulfide formation (Figure S2 and Table S1, supporting information). The significantly slower reaction with sodium azide, compared to the case where the CTA is in

the middle of the chain was an unexpected result since both DMAT and BM1429 are trithiocarbonate RAFT agents. In order to explain this phenomenon further, investigation of the relative reactivity of other CTAs with sodium azide was undertaken.

Comparison of the reactivity of different CTA types

In order to better understand which RAFT polymers are more reactive towards sodium azide, a more detailed comparison of the different CTA types was conducted. As stated above, certain dithiobenzoate CTAs have already been shown to be susceptible to nucleophilic attack at high concentrations of sodium azide.[14] In the same work, a xanthate CTA was also examined and shown to have a significantly slower reaction rate than the dithiobenzoate CTA. As far as we are aware, no investigation of CTA types has thus far been conducted and so a systematic comparison of trithiocarbonates (symmetrical and standard), dithiobenzoates, xanthates and dithiocarbamates was conducted.

A reduction in RAFT group UV absorbance over 48 hours was observed for all polymers treated with 200 ppm (3.1mM) sodium azide (**Figure 5**). For example **5.3kCPDB** and **4.6kCDMPC** were found to have a 65% and 64% reduction in CTA λ_{max} absorbance, respectively, after 48 hours. In the case of **5.3kCPDB** approximately half this reduction was recorded in the first three hours. **10kSPAT** (<7% after 48 hours) and **1kXA1** (<24% after 48 hours) experienced a relatively smaller reduction in CTA λ_{max} absorbance. This difference in reaction rate is quite significant; **5.3kCPDB** experiences a rate of attack greater than 9 times that of **10kSPAT**. Thus, for some RAFT polymers, sodium azide-containing GPC solvents will likely have a relatively insignificant effect if the sample is run within 3 hours (<2% reduction

in CTA λ_{max} absorbance for **10kSPAT** and **1kXA1**). However, other RAFT polymers (such as those with dithiobenzoate CTAs) will likely have a significant alteration to MWD, even when run within 3 hours. Overall, the results suggest that the CTA order of reactivity towards sodium azide is CPDB>>CDPMC>=DMAT>XA1>SPAT. Note that we cannot comment quantitatively on the extent of CTA removal since there may be other groups in the polymers that absorb at CTA λ_{max}.

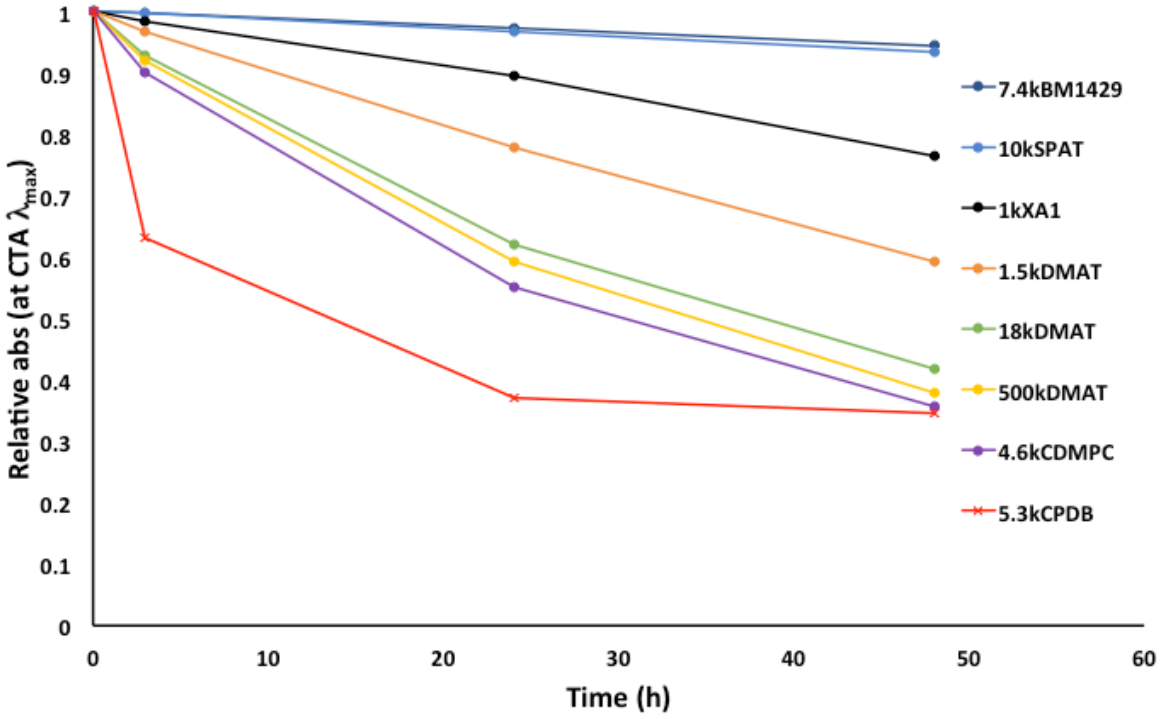

Figure 5. CTA degradation of various RAFT polymers at 0.1 mM concentration in 3.1 mM sodium azide solution. Relative absorbance is the absorbance at CTA λ_{max}/ initial absorbance at CTA λ_{max}.

In order to demonstrate the general applicability of sodium azide for CTA removal and to compare these results to the work by Wu et al.[14], a second experiment studying the rate of reaction with sodium azide at higher concentrations was also conducted. Additionally, the rate of removal was compared to that which can be achieved by methylamine at identical concentrations. The UV absorbance for each corresponding CTA was again tracked over time (**Figure 6**). During the reaction with sodium azide both **4.6kCDMPC** and **5.3kCPDB** produced a solid precipitate. To avoid erroneous results due to light scattering, the absorbance data from both of these samples has been omitted from Figure 6. The full absorbance spectrum at each time point for these samples can however been seen in Figures S3 and S4 (supporting information). ^{1}H-NMR spectra of each RAFT polymer sample before and after 48 h treatment with 267 mM sodium azide suggests complete thiocarbonyl group removal for all samples (Figures S5 – S9, supporting information). Therefore, the residual relative absorbance for each sample after 48 hours treatment with sodium azide should not be interpreted as incomplete reaction. With this in mind, almost complete CTA group removal can be achieved in 2 hours for reaction between **1.5kDMAT** and 267 mM sodium azide solution. **10kSPAT** and **1kXA1** appear to require significantly longer reaction times, with a large reduction in CTA λ_{max} absorbance occurring between 2 and 48 hours. The order of reactivity towards sodium azide was found to be consistent with the results obtained at 3.1 mM (1.5kDMAT>1kXA1>10kSPAT).

Methylamine was found to be a more effective nucleophile than sodium azide for RAFT group removal. After 2 hours of treatment with 267 mM methylamine solution the relative absorbance at λ_{max} (for CTA group) for each sample was less than 0.1. Additionally, between

2 and 48 hours of treatment little change in this absorbance was noticed. This suggests in all cases complete CTA group removal is achieved in approximately 2 hours.

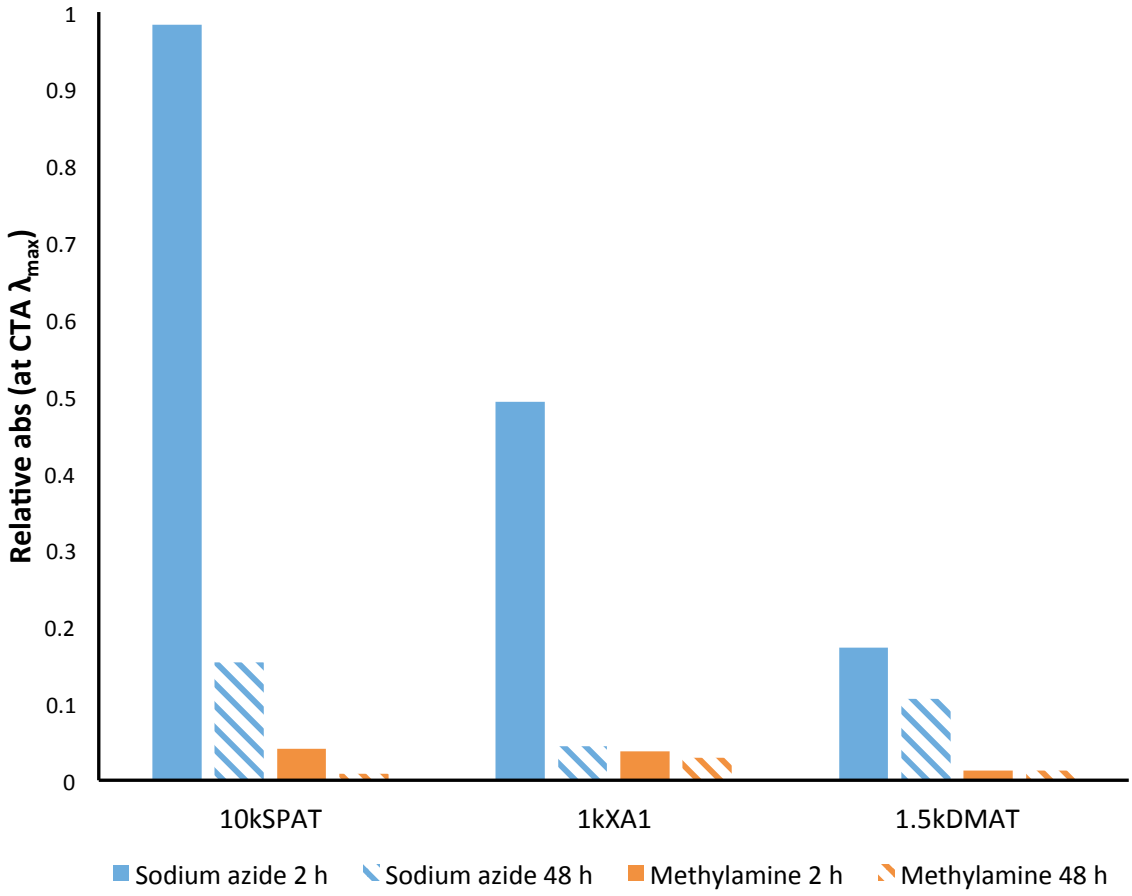

Figure 6. Relative UV-vis absorbance (at CTA λ_{max}) after 26.7 mM RAFT polymer samples have been exposed to 267 mM sodium azide (86 times the concentration used in GPC characterisation) or methylamine solution for 2 and 48 hours.

After the final UV-Vis measurement taken at 48 hours, the samples were analysed by GPC (**Table 2,** Figures S10 – S13 supporting information). **10kSPAT** and **4.6kCDMPC** polymers exposed to both methylamine and sodium azide produce polymers with a MWD shifted to higher molecular weights. Additionally, the new peak molecular weight in both cases

corresponds closely to double the M_n of the starting polymer. This shift can thus be directly explained by disulfide coupling of the polymer chains.

5.3kCPDB when reacted with sodium azide produced a less strong shift to higher molecular weights, which may be due to reaction with residual DMA monomer. For **1kXA1** a weak increase in M_n for reaction with both sodium azide and methylamine is seen, which may be simply an artefact of lower resolution for GPC analysis when analysing low molecular weight samples such as **1kXA1**.

An interesting result is seen for the **1.5kDMAT** polymer (containing a symmetrical RAFT agent); reaction with 267 mM methylamine resulted in a higher peak molecular weight than the original sample, with a comparable M_n (1.7 kDa before treatment and 1.6 kDa after) (**Figure 7**). Given that UV-vis spectroscopy measurements have shown near complete removal of the CTA, this implies near complete polymer cleavage followed by disulfide coupling of the newly formed thiol-terminated polymers. However, when **1.5kDMAT** was reacted with sodium azide a bimodal distribution was formed with one peak at the starting molecular weight and another peak at half this molecular weight. Referring to Figure 3, this suggests that the thiocarbonyl intermediate formed upon reaction of a trithiocarbonate with sodium azide is inert to further nucleophilic attack. This means that half of the chains formed after reaction were able to recombine through disulfide coupling to the original molecular weight, while the other half were inert to further reaction, remaining at half the original molecular weight.

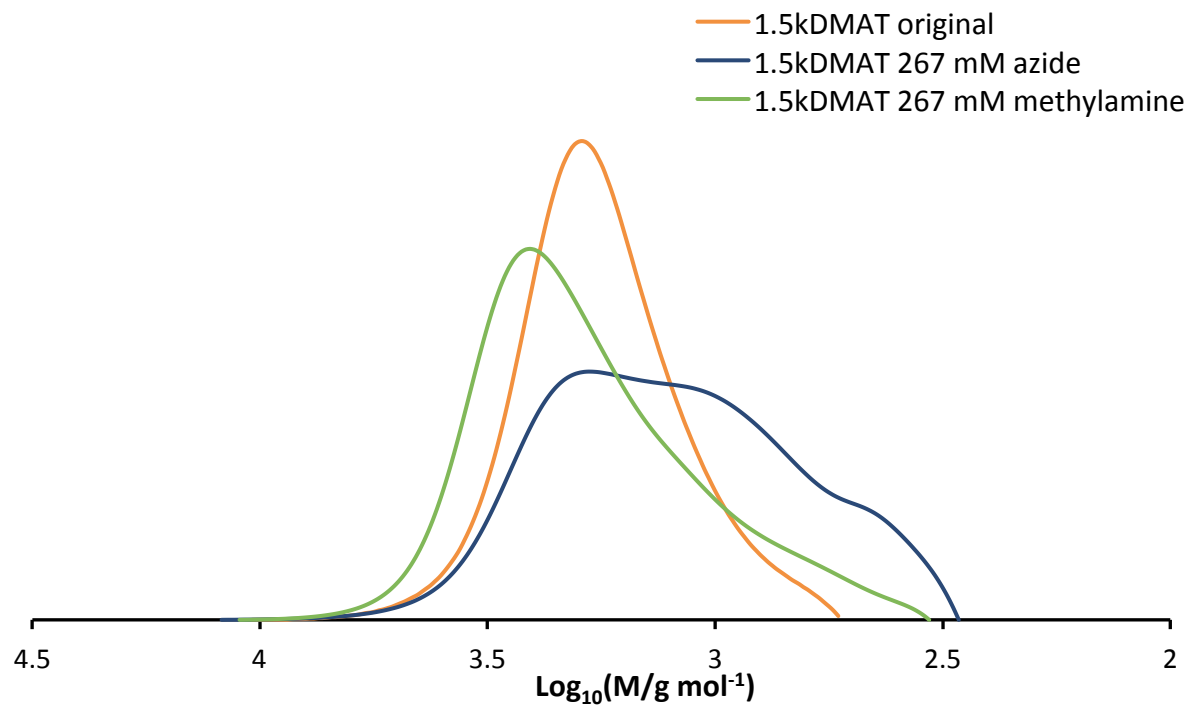

Figure 7. Changes in MWD for **1.5kDMAT** RAFT polymers after 48 hours of treatment with 267 mM sodium azide or methylamine.

Table 2. Molecular Weights and Dispersities of Polymer Samples Before and After Treatment with Nucleophile as Measured by GPC.

Polymer sample	**Original polymer**		**267 mM azide 48h**		**267mM methylamine 48h**	
	$M_{n,GPC}$ (kDa)	***Đ***	**$M_{n,GPC}$ (kDa)**	***Đ***	**$M_{n,GPC}$ (kDa)**	***Đ***
10kSPAT	7.7	1.09	9.0	1.70	11.1	1.55
4.6kCDMPC	2.8	1.15	5.5	1.24	5.6	1.34
5.3kCPDB	6.2	1.15	8.3	1.27	6.5	1.19
1kXA1	0.96	1.19	1.0	1.41	1.1	1.36
1.5kDMAT	1.7	1.17	1.0	1.46	1.6	1.35

Investigation of reaction mechanism and identification of products formed

Although it has been shown that sodium azide is capable of reacting with a range of macro-CTAs, the exact nature of the products formed is still unclear. The azide adducts shown in Figure 3 are unlikely to be stable; however, a precipitate is formed upon reaction of sodium azide with **4.6kCDMPC**, providing an opportunity to investigate further the reaction mechanism and products formed.

The white precipitate (**PX1**) collected after reaction of **4.6kCDMPC** with sodium azide was found to be unstable in solution ($CDCl_3$) at room temperature. Repeated analysis over time by ^{1}H-NMR and ^{13}C-NMR spectroscopies demonstrated the gradual formation of a second, solution-stable product (**PX2**) (**Figure 8** and **Figure S14**). After approximately 6 days **PX1** had been completely converted to **PX2**. The emergence of a carbon environment at 106.5 ppm in **PX2** was interpreted as the formation of a nitrile on what previously appeared to be the thiocarbonyl carbon. This, along with NMR peaks corresponding to a pyrazole group, was then used to generate the proposed structures shown in **Figure 9.** These structures were supported by mass spectrometry performed on the **PX1** sample (Figure S15, supporting information). Given that **PX1** is unstable at room temperature, it is not surprising that significant fragmentation to **PX2** appears to have occurred during MS analysis (calculated for $C_6H_7N_3$ +1H^+ 122.0718, found 122.0714). Additionally, if the proposed structures are correct, the transition from **PX1** to **PX2** would involve the release of a nitrogen molecule and elemental sulfur. Evidence for the formation of N_2 and sulfur is seen in both the MS spectra (loss of 28.0064 and 31.972 between main ions) and through the formation of an insoluble yellow by-product. These results are in excellent agreement with previous literature studies on the formation of 1,2,3,4-thiatriazoles by reaction of dithioesters with sodium azide.[21]

Thiatriazoles are known to be unstable compounds in solution at room temperature and degrade into nitrogen gas, sulfur and a nitrile.[21-23] However, the formation of a thioacyl azide (as proposed in the work by Wu et al. [14]) instead of a thiatriazole cannot be ruled out from the above evidence since it will produce MS and NMR spectra in line with what has been observed experimentally. Importantly, it is known that the ring structure of thiatriazoles does not have an IR absorbance at 2100 – 2200 cm^{-1}, while a thioacyl azide would since the azide absorbance at 2100 – 2200 cm^{-1} is so strong and little altered by surrounding groups.[24] IR analysis performed on **PX1** gave no clear peak above 1600 cm^{-1}, confirming the thiatriazole structure. A nitrile peak at 2251cm^{-1} for **PX2** was observed, confirming its proposed structure (Figure S16, supporting information). Additionally, an IR absorption at 1583cm^{-1} was observed and is in line with reported values for the C=N and N=N stretching vibrations for the heteroaromatic ring system.[23]. Thus, it is proposed that sodium azide reacts with the CTA on the 4.6kCDMPC polymer chain producing a thiol-terminated polymer and the unstable 1,2,3,4-thiatriazole. This then degrades in solution at room temperature into nitrogen gas, elemental sulfur and a nitrile attached to the Z group of the CTA (**Figure 9**).

The solid precipitate (**PY1**) from reaction of sodium azide with **5.3kCPDB** was similarly analysed by mass spectrometry and FTIR which indicated that another thiatriazole had formed (Figures S17 – S18, supporting information).

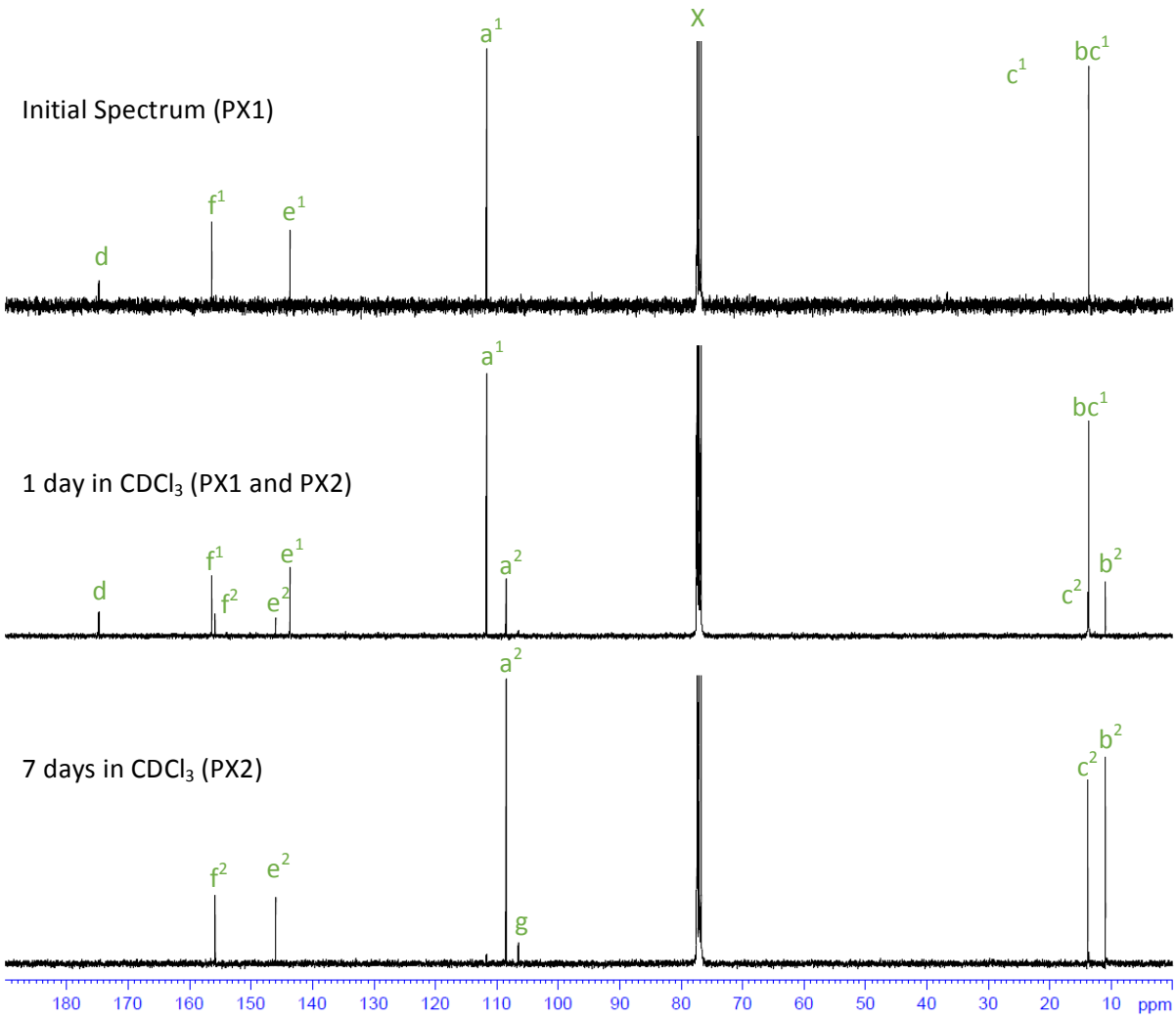

Figure 8. ^{13}C-NMR spectrum ($CDCl_3$) over time of precipitated solid from sodium azide reaction with **4.6kCDMPC.**

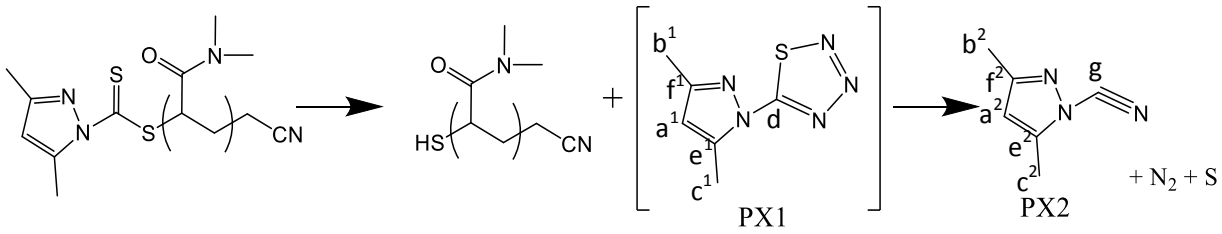

Figure 9. Proposed reaction scheme for **4.6kCDMPC** RAFT group removal by reaction with sodium azide. **PX1** is the initially formed unstable precipitate, **PX2** is its stabilised degradation product.

Although not demonstrated here, it does appear likely that all CTAs form a thiatriazole intermediate followed by a nitrile upon reaction with azide. The reaction of azide with dithiobenzoates[25] and compounds similar to xanthates[26], dithiocarbamates[27] and trithiocarbonates[28, 29] to produce corresponding (unstable) thiatriazoles has been reported. Lastly, the lack of thioacyl azide compounds reported in the literature to date adds further weight to this argument (currently thiobenzoyl azide S-oxide is the only thioacyl azide-like structure observed).[30]

Conclusions

Aqueous GPC solvents containing 200 ppm sodium azide can react with RAFT polymers causing alterations to the MWD. Preparation of RAFT polymer samples in a solvent without sodium azide is recommended to minimise the exposure time of the polymer to this nucleophile. The rate of sodium azide attack on RAFT polymers was found to vary significantly between CTAs with up to 9 times difference in rate of cleavage observed. Additionally, the use of sodium azide as an alternative to amines for RAFT group removal was demonstrated, however its removal rate was found to be slower than when identical molar concentrations of methylamine were used. The reaction of sodium azide with RAFT agents produces an unstable thiatriazole-functionalised Z group and a thiol terminated

polymer. The thiatraizole then degrades further to form a nitrile-functionalised Z group, N_2 and sulfur.

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References

1. J. Chiefari, Y. K. Chong, F. Ercole, J. Krstina, J. Jeffery, T. P. T. Le, R. T. A. Mayadunne, G. F. Meijs, C. L. Moad, G. Moad, E. Rizzardo and S. H. Thang, *Macromolecules*, 1998, **31**, 5559-5562.
2. G. Moad, E. Rizzardo and S. H. Thang, *Aust. J. Chem.*, 2005, **58**, 379-410.
3. G. Moad, E. Rizzardo and S. H. Thang, *Aust. J. Chem.*, 2012, **65**, 985-1076.
4. M. R. Hill, R. N. Carmean and B. S. Sumerlin, *Macromolecules*, 2015, **48**, 5459-5469.
5. D. B. Thomas, A. J. Convertine, L. J. Myrick, C. W. Scales, A. E. Smith, A. B. Lowe, Y. A. Vasilieva, N. Ayres and C. L. McCormick, *Macromolecules*, 2004, **37**, 8941-8950.
6. A. B. Lowe and C. L. McCormick, *Prog. Polym. Sci.*, 2007, **32**, 283-351.
7. P. E. Millard, L. Barner, J. Reinhardt, M. R. Buchmeiser, C. Barner-Kowollik and A. H. E. Muller, *Polymer*, 2010, **51**, 4319-4328.
8. L. Martin, G. Gody and S. Perrier, *Polym. Chem.*, 2015, **6**, 4875-4886.

9. E. Read, A. Guinaudeau, D. J. Wilson, A. Cadix, F. Violleau and M. Destarac, *Polym. Chem.*, 2014, **5**, 2202-2207.

10. R. N. Carmean, T. E. Becker, M. B. Sims and B. S. Sumerlin, *Chem*, 2017, **2**, 93-101.

11. L. Albertin, A. Wolnik, A. Ghadban and F. Dubreuil, *Macromol. Chem. Phys.*, 2012, **213**, 1768-1782.

12. M. Obata, T. Kobori, S. Hirohara and M. Tanihara, *Polym. Chem.*, 2015, **6**, 1793-1804.

13. O. O. Oyeneye, W. Z. Xu and P. A. Charpentier, *RSC Adv.*, 2015, **5**, 76919-76926.

14. Y. Wu, Y. Y. Zhou, J. Zhu, W. Zhang, X. Q. Pan, Z. B. Zhang and X. L. Zhu, *Polym. Chem.*, 2014, **5**, 5546-5550.

15. C. J. Ferguson, R. J. Hughes, D. Nguyen, B. T. T. Pham, R. G. Gilbert, A. K. Serelis, C. H. Such and B. S. Hawkett, *Macromolecules*, 2005, **38**, 2191-2204.

16. J. T. Lai, D. Filla and R. Shea, *Macromolecules*, 2002, **35**, 6754-6756.

17. M. Destarac, C. Brochon, J. M. Catala, A. Wilczewska and S. Z. Zard, *Macromol. Chem. Phys.*, 2002, **203**, 2281-2289.

18. J. Xu, J. He, D. Fan, X. Wang and Y. Yang, *Macromolecules*, 2006, **39**, 8616-8624.

19. X. P. Qiu and F. M. Winnik, *Macromol. Rapid Commun.*, 2006, **27**, 1648-1653.

20. Q. L. Zhang, L. Voorhaar, B. G. De Geest and R. Hoogenboom, *Macromol. Rapid Commun.*, 2015, **36**, 1177-1183.

21. K. A. Jensen and C. Pedersen, *Adv. Heterocycl. Chem.*, 1964, **3**, 263-284.

22. W. Dehaen and V. A. Bakulev, in *Comprehensive Heterocyclic Chemistry III*, eds. C. A. Ramsden, E. F. V. Scriven and R. J. K. Taylor, Elsevier, Oxford, 2008, pp. 441-484.

23. A. Holm, *Adv. Heterocycl. Chem.*, 1976, **20**, 145-174.

24. E. Lieber, C. N. Pillai, J. Ramachandran and R. D. Hites, *J. Org. Chem.*, 1957, **22**, 1750-1751.

25. K. A. Jensen and C. Pedersen, *Acta Chem. Scand.*, 1961, **15**, 1104-1108.

26. D. Martin, *Tetrahedron Lett.*, 1964, 2829-2832.

27. G. Mloston, M. Woznicka and H. Heirngartner, *Helv. Chim. Acta*, 2007, **90**, 594-600.

28. D. Martin, *Chem. Ber. Recl.*, 1964, **97**, 2689-2694.

29. H. C. Hansen and A. Senning, *J. Chem. Soc., Chem. Commun.*, 1979, 1135-1136.

30. A. Holm, in *Comprehensive Heterocyclic Chemistry*, ed. C. W. Rees, Pergamon, Oxford, 1984, pp. 579-612.