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Hydrolysis of hydrophobic poly(2-oxazoline)s and their subsequent modification *via* aza-Michael addition

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ABSTRACT

Partially hydrolysed poly(2-oxazoline)s possess unique properties. However, much of the focus in this area has been on water soluble poly(2-oxazoline)s. Where hydrophobic poly(2-oxazoline)s have been used, this is often for selective hydrolysis. However, hydrolysis of very hydrophobic polymers could lead to interesting solution behaviour. Herein, we describe universal conditions for the hydrolysis of poly(2-alkyl-2-oxazoline)s suitable for both hydrophobic and hydrophilic 2-oxazolines. We show that the system utilised gives comparable rates to that of water alone for poly(2-ethyl-2-oxazoline). In addition, poly(2-fatty acid-2-oxazoline) was hydrolysed using the developed system and was found to proceed in a controlled manner allowing the targeting of specific degrees of hydrolysis, albeit much slower than for poly(2-ethyl-2-oxazoline). Finally, we demonstrate the partial functionalisation of poly (2-oxazoline)-poly(ethylene imine) co-polymers *via* aza-Michael addition.

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KEYWORDS
Poly(2-oxazoline); cationic ring-opening polymerization; hydrolysis of polymers; poly(ethylene imine)

Introduction

Poly(2-oxazoline)s are important bio-medically relevant polymers formed from the cationic ring-opening polymerization (CROP) of 2-oxazolines owing to their improved performance over common biomedical polymers such as poly(ethylene glycol) [1–4]. Notwithstanding the range of commercial monomers available, a number of functional monomers have been reported in the literature [5]. However, monomer design is limited in that nucleophilic side groups have to be avoided which limits the synthetic possibilities of poly(2-oxazoline)s. Therefore, a number of post-polymer modification strategies such as click reactions to functional monomers [6–10], polymer–polymer coupling reactions [11], star polymers via click reactions [12–14], cyclisation [15–21], brush polymers [22–24] as well as hydrolysis [25,26] have been used to increase the functionality and/or create novel macromolecular architectures. In particular, both acidic and basic hydrolysis has received a lot of attention due to the controlled and facile nature of the process in forming poly(2-oxazoline) (POx)/poly(ethylene imine) (PEI) co-polymers [27,28]. Moreover, POx-PEI copolymers show good RNA and DNA transfection with the ability to tune the hydrolysis being of critical importance because the charge density, molecular weight and amount of PEI all impact the properties of the co-polymers [24,29–34]. In addition, comb PEI structures have also been developed utilising the controlled hydrolysis of poly(2-oxazoline)s [32,35]. The hydrolysis of poly (2-ethyl-2-oxazoline) and poly(2-methyl-2-oxazoline) has been extensively studied with numerous studies on the mechanism and kinetics of the hydrolysis [25,29,36]. Indeed, selective hydrolysis of hydrophilic blocks has been demonstrated, allowing the altering of properties, such as solution behaviour [37–39].

Moreover, several post-hydrolysis functionalisation techniques have been explored to further increase the functionality [28]. This allows functional groups to be included in the polymer that would have otherwise been prohibited on account of their CROP interfering properties. Examples of these include acylation [40–42], nucleophilic substitutions to alkyl halides [24] and reductive amination [43]. In addition, Michael additions to linear PEI have been reported [44].

In this study, we report on the kinetics of the hydrolysis of a very hydrophobic poly(2-oxazoline) possessing C17 alkyl chains (PFAOx) in a THF/water co-solvent mixture. In addition, we have applied these conditions to poly(2-ethyl-2-oxazoline) (**PEtOx**) alongside aqueous systems to measure the impact of this solvent system on the kinetics. Finally, we demonstrate the partial aza-

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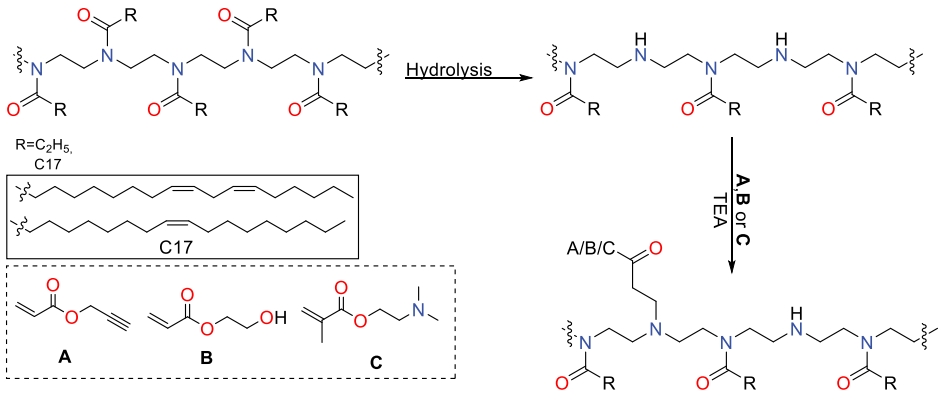


Scheme 1. Overall reaction scheme showing the partial hydrolysis of poly(2-oxazoline)s followed by the subsequent aza-Michael addition.

Michael functionalisation of partially hydrolysed poly (2-oxazoline)s with selected vinyl monomers (Scheme 1).

Experimental

Materials

Hydrochloric acid (Fisher, 37%), thiophenol (Fisher, 99%), azobisisobutyronitrile (AIBN, Sigma Aldrich, 98%), propargyl acrylate (Sigma, 98%), 2-hydroxyethyl acrylate (Sigma, 96%), 2-(dimethylamine)ethyl methacrylate (Sigma, 98%) and 1,4-butanediol (Sigma-Aldrich, 98%) were all used as received.

Instruments

Most GPC chromatograms were measured on an Agilent Technologies 1260 Infinity fitted with a refractive index detector, a PLgel 5 µm guard column and a PL gel 5 µm mixed D column (300 × 7.5 mm). THF with 2% triethylamine was used as the eluent. Samples were run at 40 °C with a flow rate of 1 mL min^{-1} and measured against narrow poly(methyl methacrylate) standards. Figure 3 was measured in a similar way but employed 2× PL gel 5 µm mixed D columns.

All ^{1}H NMR were measured at 298 K on a Bruker HD300 or HD400 in $CDCl_3$.

Procedure for the hydrolysis kinetic of PFAOx

PFAOx (3.8880 g) was dissolved in THF (21 mL) and the resulting stock solution divided into microwave vials in 1.6 mL portions. Thirty-five percent hydrochloric acid (0.4 mL) was added, and the resulting solution reacted at 120 °C for the indicated time. At the end of the reaction, the solution was precipitated into acetone. The resulting solids were then dissolved in THF and neutralised to a pH <9 with ~4 M NaOH(aq). The THF was removed in *vacuo* with the resulting water removed by freeze drying. To analyse the samples, the polymer was dissolved out of the residue using deuterated chloroform (for ^{1}H NMR) or THF/2% TEA (for GPC).

Procedure for the hydrolysis kinetics of PEtOx

PEtOx (1.0033 g) was dissolved in THF (17 mL) and the resulting stock solution was divided into microwave vials in 1.6 mL portions. Thirty-five percent hydrochloric acid (0.4 mL) was added, and the resulting solution reacted at 120 °C for the indicated time. At the end of the reaction, the solution was neutralised to a pH <9 with ~4 M NaOH(aq). The THF was removed in *vacuo* with the resulting water removed by freeze drying. The samples were dissolved in deuterated methanol for analysis. The kinetics for the other systems used were identical, replacing THF with water and 1,4-butanediol, as appropriate.

Procedure for the aza-Michael addition to hydrolysed poly(2-oxazoline)s

A typical procedure was as follows: The polymer was dissolved in THF to a concentration of 100 mg/mL and the appropriate amounts of monomer and TEA were added (see Table 1). The reaction was reacted at the temperature and time indicated in Table 1. Where purified, the **PFAOx** Michael addition conjugates were precipitated in methanol, and the **PEtOx** conjugates were precipitated in diethyl ether.

Procedure for the thiol-yne reaction

PE4 (25.2 mg, 1 eq.) was dissolved in DMF (0.75 mL) along with AIBN (2.2 mg, 59 mol%) and purged with nitrogen. Thiophenol (0.03 mL, excess) was added, and

Table 1. Summary table for the conditions studied for the aza-Michael additions carried out for **PEtOx** (**PE1-PE7**) and **PFAOx** (**PF1-PF4**.).

Run	Monomer	Equivalents of Base	Equiv. of Monomer	T (°C)	Time (hours)	Functionalisation (%)
PE1	A	1.5	1.5	60	15	20[a]
PE2	A	1.5	1.5	120	2	27[a]
PE3	A	PE2 (+1.5)	PE2 (+1.5)	120	PE2(+2)	41[a]
PE4	A	1.5	1.5	120	15	59[a]
PE5	A	5	5	140	5	59[a]
PE6	B	1.5	1.5	120	2	21[b]
PE7	C	1.5	1.5	120	2	4[b]
PF1	A	3	1.5	120	2	56[a]
PF2	A	3	1.5	130	2	58[a]
PF3	A	3	1.5	120	16	60[a]
PF4	A	5	3	130	2	58[a]

[a]Functionalisation calculated according to the ratio of -CH_2- of the propargyl group to the hydrolysis peak (equation 3), [b]Functionalisation calculated by measuring the reduction in the hydrolysis peak. All reactions were carried out in THF with TEA as base.

the reaction was left for 15 h at 90 °C. The reaction was precipitated in diethyl ether before analysis.

Results and discussion

Poly(2-oxazoline) hydrolysis kinetics

Figure 1a shows the hydrolysis kinetics for both **PFAOx** with a [M]:[I] of 50:1 and several conditions for **PEtOx** with a [M]:[I] of 46:1. Given the solubility in water of **PEtOx,** the hydrolysis has been reported to be carried out in aqueous hydrochloric acid at various temperatures, leading to well controlled partially hydrolysed poly(ethyl oxazoline)-poly(ethyleneimine) copolymers [26,29]. **PFAOx** being inherently hydrophobic is non-water soluble and, therefore, the hydrolysis cannot proceed in a purely aqueous environment. Given this, a tetrahydrofuran (THF)/HCl_{aq} 8:2 co-solvent mixture was employed, which assuming complete and thorough mixing of solvent gives an acid concentration of 2.4 M. The amide concentration used was 0.48 M calculated using an average repeating unit molecular weight of 306 Da and 99 Da for **PFAOx** and **PEtOx**, respectively.

Upon dissolution of **PFAOx** in THF, a phase separation between the organic and aqueous layers is observed at room temperature. Due to this phase separation, a stock solution using both acid and THF was not possible. To allow proper comparison, all other kinetic sequences discussed herein used the same set-up method and therefore any error should be consistent. However, upon heating no phase separation is observed, although upon cooling the separation returns. At the completion of the reactions, the samples were prepared (see experimental) and the ^{1}H NMR was measured to obtain the degree of hydrolysis. In the ^{1}H NMR, the hydrolysis peak overlaps with a side chain peak on the spectrum (see Figure S1, ESI) which means a direct measurement could not be obtained. Therefore, given that the ratio of this peak to the other side chain peaks remains constant at any given degree of hydrolysis, any increase in the integration of this peak can be

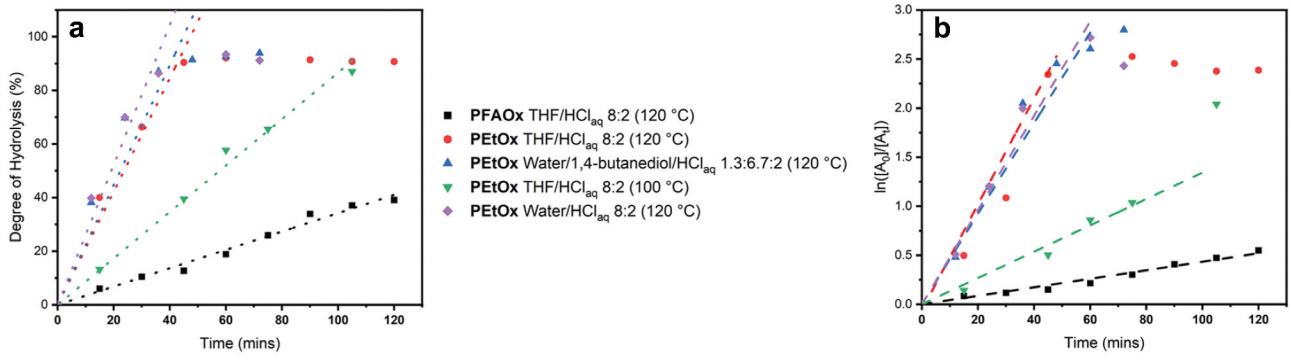


Figure 1. a) hydrolysis kinetics showing **PFAOx** hydrolysed in 8:2 THF/HCl_{aq} (black), **PEtOx** hydrolysed in 8:2 THF/HCl_{aq} (red), **PEtOx** hydrolysed in 6.3:1.3:2.4 1,4-butanediol/water/HCl_{aq} (blue), **PEtOx** hydrolysed in 8:2 THF/HCl_{aq} at 100 °C (green) and **PEtOx** hydrolysed in 8:2 water/HCl_{aq} (purple). b) corresponding first-order kinetic plot. All reactions were carried out with 35–37% HCl_{aq} such that [H^+]=2.4 M and [A]=0.48 M. **PFAOx** hydrolysis was calculated according to Equation 1 and all **PEtOx** hydrolysis rates were calculated according to Equation 12.

attributed to the hydrolysis. Hence, the hydrolysis was calculated according to equation Equation 1, setting the integral value for one of the side chain peaks to the same value in both t_{final} and t_0:

$$\textit{Degree of Hydrolysis}\,(\%) = 100\left(\frac{I_{H,tf} - I_{H,t0}}{I_P}\right) \quad (1)$$

Where $I_{H,tf}$ is the integral for the hydrolysis co-peak at t final, $I_{H,t0}$ is the integral for the hydrolysis co-peak at t_0 and I_P is the integral for the main polymer backbone peak.

As can be seen from Figure 1, the hydrolysis proceeds in a controlled fashion with the hydrolysis increasing linearly with time. This would allow specific hydrolysis rates to be targeted which could have interesting solubility behaviour.

For comparison, the hydrolysis of **PEtOx** was also carried out in THF/water using the same conditions as for **PFAOx**. It is important to note that the target molecular weights of both polymers were different (although the [M]:[I] ratios were approximately equal), however, Hoogenboom *et al.* [26] found no molecular weight dependency on the hydrolysis kinetics of **PEtOx**. The appearance of a second peak that overlaps with the non-hydrolysed backbone peak means that this peak could not be used. As hydrolysis progresses the overlap is absent because of a much smaller amount of non-hydrolysed backbone and thus a smaller peak is observed for that species. This extra peak is due to the hydrolysis of THF forming 1,4-butanediol as a side reaction (see Figure S2, ESI). Nevertheless, the non-hydrolysed side chains were used, according to Equation 2. For accurate comparisons, all other **PEtOx** kinetics also utilised equation 2.

$$\textit{Degree of hydrolysis}\,(\%) = 100\left(\frac{I_H}{2I_{CH2} + I_H}\right) \quad (2)$$

Where I_H is the integral for the hydrolysis peak and I_{CH2} is for the integral of the remaining CH_2 groups on the polymer chain.

As can be seen from the figure, **PEtOx** experiences significantly faster hydrolysis under THF/HCl_{aq} 8:2 conditions compared to **PFAOx** with ca. 90% hydrolysis being reached in 45 min compared to 14% hydrolysis for **PFAOx**; an over 6-fold difference. Interestingly, the polymer precipitated out of the reaction solution upon cooling down due to the high number of positive charge which make the polymer insoluble in THF. This significant difference in rate is because the rate determining step in the hydrolysis is the nucleophilic addition of water [28]. With **PFAOx**, the more sterically hindered, hydrophobic polymer chains cause the rate of this nucleophilic attack to be dramatically lowered thus leading to an overall lower hydrolysis rate. In addition, the kinetic for **PEtOx** using THF/HCl_{aq} 8:2 was carried out at 100 °C. As can be seen from Figure 1, the rate of hydrolysis is lower than at 120 °C, as expected. Highlighting the slower rate of **PFAOx** hydrolysis, even at 100 °C the hydrolysis of **PEtOx,** is faster than that of **PFAOx** at 120 °C under the same conditions.

As previously mentioned, there was evidence of THF hydrolysis in the reaction of **PEtOx** at 120 °C in THF/HCl_{aq} 8:2. In order to investigate if this side reaction had an impact on the kinetics, a kinetic experiment was carried out, but the THF/HCl_{aq} 8:2 solvent mixture was replaced with 1,4-butanediol/water/HCl_{aq} 6.7:1.3:2 with [A] = 0.48 M. As can be observed from Figure 1a, a very slight acceleration in the rate is observed, but this is not significant and is virtually the same as the THF/HCl_{aq} mixture. Similarly, the kinetic was also carried out in water under the same acid and amide concentrations, and the hydrolysis was observed to proceed in the same manner as the THF/HCl_{aq} 8:2 mixture. These results suggest that a THF/HCl_{aq} mixture gives similar hydrolysis behaviour to water alone, which is important given the poor suitability in aqueous solutions for very hydrophobic poly(2-oxazoline)s.

To verify the results obtained from Equation 2, other methods were also carried out. For the hydrolysis of **PEtOx** in water two equations, commonly used in the literature [45], were used in addition to Equation 2. These results are plotted and shown in the supplementary information (Figure S3). In general, there was good agreement between all three equations used differing by only a few percent. At higher hydrolysis, the difference was greater but still within approximately 5%.

The hydrolysis of poly(2-oxazoline)s have been reported to proceed in a first-order fashion [26]. Therefore, the first-order plot for the linear portions of the hydrolysis kinetics was plotted for all conditions used and is displayed in Figure 1b. From the slopes, the hydrolysis rate constant, k_h^{app}, can be obtained. The results show that the rate of hydrolysis for **PFAOx** is approximately 10 times slower than **PEtOx**, under the same conditions. The data also shows that the rate of hydrolysis is very similar for all conditions used, further indicating the suitability of THF/water systems for the hydrolysis of poly(2-oxazoline)s.

Figure 2a shows the GPC chromatograms for the **PFAOx** kinetic and shows the reduction in apparent molecular weight as the hydrolysis progresses. As expected, a low molecular weight shoulder is evident and increases as the degree of hydrolysis increases. The origin of the low molecular weight tailing is due to increased column interactions as the level of hydrolysis increases. Due to the tailing making integration difficult,

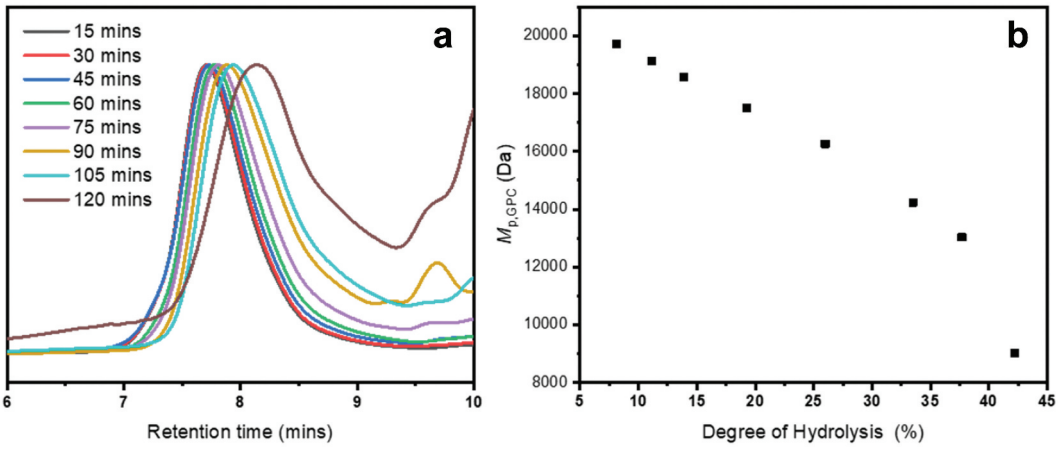


Figure 2. a) GPC chromatograms for the hydrolysis of **PFAOx**, showing the shift to lower molecular weight and the increase in tailing at higher degrees of hydrolysis. b) plot of $M_{p,GPC}$ with degree of hydrolysis.

rather than using $M_{n,GPC}$ the $M_{p,GPC}$ has been used and was plotted along with the degree of hydrolysis (Figure 2b). As can be observed, the $M_{p,GPC}$ decrease in a linear fashion as the degree of hydrolysis increases. However, the $M_{p,GPC}$ is far lower than would be expected for the 120-min data point. One potential reason for this could be reduced solubility in THF of the hydrolysed material as hydrolysis increases. Notwithstanding this, taken together, the data suggests that THF/HCl_{aq} mixtures are effective for the hydrolysis of both hydrophilic and hydrophobic poly(2-oxazoline)s.

Aza-Michael addition of vinyl monomers to Poly (2-Oxazoline)s

The acylation of (partially) hydrolysed **PEtOx** has been reported in order to create poly(oxazoline)s with functional groups that otherwise would not be possible from a monomeric route [41,42]. Here, the aza-Michael addition of the PEI secondary amines to vinyl monomers was investigated for **PEtOx** and **PFAOx** to provide a route to introducing additional functionality.

PE1-PE7 used a poly(2-ethyl-2-oxazoline) polymer with a targeted [M]:[I] = 100:1 and a targeted 10–13% hydrolysis rate. Although only necessary for the calculation of **PE6** and **PE7,** the starting polymer for all entries except **PE1** and **PE3** had a hydrolysis rate of 13.1%, with the other hydrolysed polymer having a hydrolysis rate of 11.5%. All hydrolysis was performed in the same way as Hoogenboom *et al*. [26] utilising the kinetic they obtained at 100 °C in water. All equivalents of base and monomer were calculated using the theoretical molecular weight including the degree of hydrolysis.

$$\%Functionalisation = \frac{2I_{CH_2}}{I_H + 2I_{CH_2}} \quad (3)$$

Where I_{CH2} is the integral for the CH_2 at 4.5 ppm adjacent to the triple bond and I_H is the integral for the hydrolysis peak

For **PE1-PE5** (Table 1), monomer **A** (propargyl acrylate) was used because the -CH_2- adjacent to the triple bond has a clear and unobstructed peak in the ^{1}H NMR at 4.5 ppm. This peak was used to calculate the amount of functionalisation after the Michael addition by calculating the ratio between the peak and the hydrolysis backbone peak (see Figure S4, ESI). The main advantage of this method is that the degree of hydrolysis does not need to be known and this method is independent of molecular weight. The importance of this will become more apparent when **PFAOx** is discussed. **PE1-PE4** all used 1.5 equivalents of monomer and base with the only difference being to change the reaction time and/or temperature. Carried out at 60 °C for 15 h, **PE1** shows a functionalisation of 20%. Conversely, **PE2** was reacted at double the temperature for a fraction of the time (2 h) and shows a higher functionalisation of 27% which suggests temperature has an effect. **PE2** was then purified and re-reacted with 1.5 equivalents of both base and monomer **A** and reacted again for 2 h at 120 °C (**PE3**). As can be seen in Table 1 the degree of functionalisation increases from 27% to 41%. The effect of leaving the reaction at high temperatures for 15 h was also investigated (**PE4**) and the functionalisation increased from 27% to 59% going from 2 h to 15 h. Forcing conditions of 140 °C, 5 equivalents of monomer and base for 5 h (**PE5**) was also investigated, and the functionalisation was found to be 59%, the same as for **PE4**.

In addition to monomer **A**, two other monomers were also investigated to look at the substrate scope of these

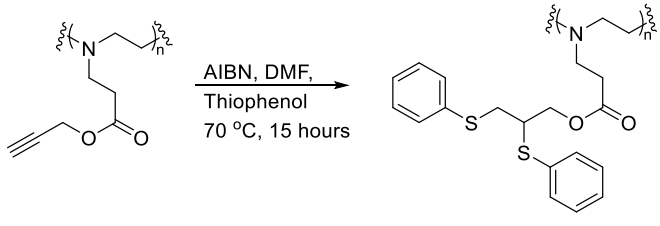
reactions. In the same way as monomer **A**, for monomer **B** (hydroxyethyl acrylate) the – CH_2- peaks adjacent to the OH could be used to calculate the ratio between monomer B and the hydrolysed backbone peak and therefore the functionalisation. The same conditions were used as **PE2** using monomer **B** (**PE6**) and the functionalisation was found to be 21% some 6% lower than when using monomer **A**. This suggests that the nature of the Michael acceptor is a determining factor in the amount of functionalisation, as expected. Moreover, the functionalisation was also measured when a methacrylate (monomer **C**) was utilised, and the functionalisation found to be significantly lower at 4% (**PE7**). The drop in functionalisation is likely due to methacrylates being poorer Michael acceptors due to the σ-donation of the methyl group. It is important to note, though, that unlike with monomer **A,** no clear and unobstructed peak in the ^{1}H NMR exists for monomer **B** and therefore an alternative calculation was required. In this case, the reduction in the hydrolysis peak was used. This method is less favourable because a 1% difference on the hydrolysis calculation leads to a 10% difference in the functionalisation for a polymer with a hydrolysis level of 10%.

Discussion now turns to **PF1-PF4** where **PFAOx** was used. For these polymers, the starting polymer had a targeted [M]:[I] = 50:1 and a targeted 24% hydrolysis rate formed using the THF/water 8:2 system developed here. The starting polymer was used directly after the hydrolysis with the exception of a precipitation in acetone step. Therefore, the polymer was still the hydrochloride salt, and hence, an elevated amount of TEA was used. As can be seen from Table 1, all entries have a similar level of functionalisation suggesting that a ceiling is reached beyond which the functionalisation cannot proceed. It is likely that steric factors and conformation in solution play a role in preventing further functionalisation. Additionally, equivalents of base or monomer were not found to impact the functionalisation. It is worth noting the contrast between **PF1** and **PE2**, whereby similar conditions were employed. **PF1** shows significantly more functionalisation which is suggestive of a conformational role given that the steric hindrance in the case of **PFAOx** is far higher. Alternatively, the sterically hindered chains could encourage hydrolysis on adjacent amides, allowing a more block-type architecture to form lowering steric hindrance and making functionalisation more facile.

Thiol-yne on PEtOx-monomer A conjugate

To further highlight the scope of reaction, a monomer **A** conjugate, **PE4**, was reacted in a thiol-yne reaction with thiophenol. A large excess (10 equivalents) with respect to the alkyne group, as calculated using the theoretical molecular weight, with AIBN being used as catalyst (see scheme 2). The ^{1}H NMR of **PE4** and the crude material after reaction are shown in Figure 3. The data indicates the complete loss of the singlet peak at 4.65 ppm which is indicative of the – CH_2 adjacent to the triple bond. In a thiol-yne 2 thiol additions are possible with one addition leading to the formation of a double bond, with the second addition being significantly faster [46]. In Figure 3a, the ^{1}H NMR before (**PE4**, red) and after the thiophenol addition (blue) is shown. It provides clear evidence that the thiol-yne reaction proceeded in a quantitative manner with all propargyl groups reacting, as evidenced by the disappearance of the CH_2 adjacent to the triple bond (light blue circle). Moreover, the presence of the post-reaction peaks (grey, black and green circles) further confirms that the addition has proceeded in the expected fashion. There is no evidence of double bond formation indicating that a complete bis-addition has occurred.

Additionally, the GPC shown in Figure 3b, shows a shift to higher molecular weight consistent with addition. The disappearance of the high molecular weight shoulder in **PE4**, an artefact from the initial starting polymer, is noted. Having established that the alkyne group is still accessible and reactive to thiols, there are no obvious reasons that copper catalysed azide alkyne cycloadditions cannot also be performed to further functionalise the material potentially using azide terminated polymers.



Scheme 2. Partial reaction scheme for the thiol-yne click reaction of PE4.

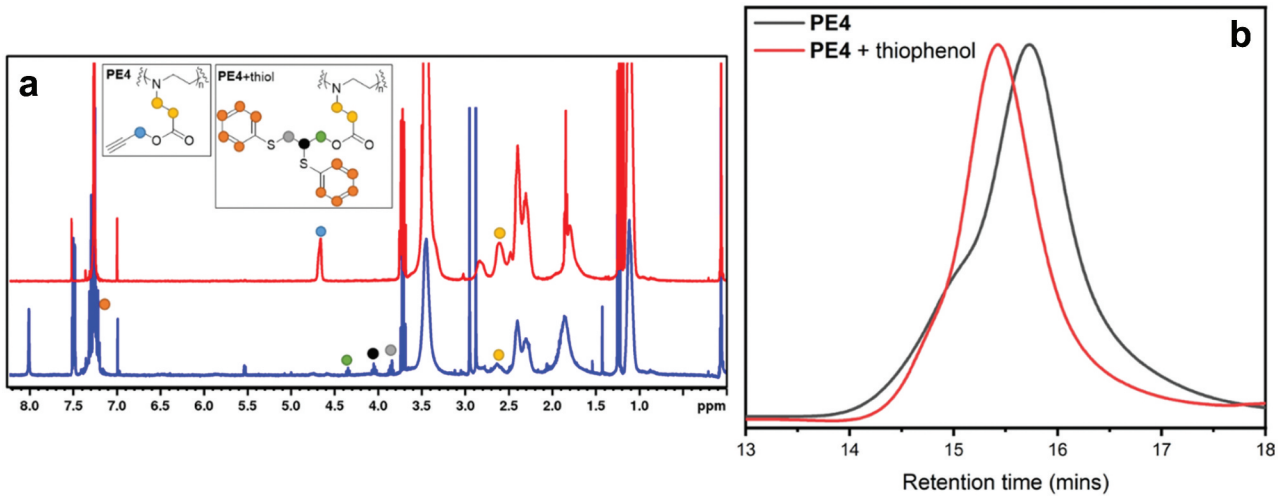


Figure 3. 1H NMR spectrum of **PE4** before and after thiol-yne click reaction (a), and the GPC traces (RI detector) of **PE4** before and after reaction with thiophenol (b).

Conclusion

In conclusion, a universal hydrolysis system suitable for any poly(2-oxazoline) has been developed and the rate of hydrolysis shown to be comparable to the commonly used aqueous systems. The hydrolysis of long chained, hydrophobic poly(2-fatty acid-2-oxazoline) was found to proceed at a vastly reduced rate due to steric hindrance and hydrophobicity. In addition, the aza-Michael addition of propargyl acrylate was also demonstrated with **PFAOx** and **PEtOx** reaching 59% and 60% functionalisation, respectively. In addition, 2-hydroxyethyl acrylate was found to proceed in a comparable manner to that of propargyl acrylate whereas 2-(dimethylamino)ethyl methacrylate was observed to have a much lower functionalisation of 4%. Finally, the aza-Michael conjugate of **PEtOx** and propargyl acrylate was used in a thiol-yne click reaction with the reaction proceeding to completion. The future would include looking into the solution behaviour of partially hydrolysed **PFAOx**.

Acknowledgments

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

FURTHER DATA WILL BE AVAILABLE UPON REQUEST.

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