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Fast and Accurate Partial Hydrolysis of Poly(2-ethyl-2-oxazoline) into tailored Linear Polyethylenimine Copolymers

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Partial hydrolysis of poly(2-oxazoline)s yields poly[(2-oxazoline)-*co*-(ethylenimine)] copolymers that are of interest for a broad range of applications, from switchable surfaces, nanoparticles and hydrogels, to gene delivery and biosensors. In the present research, a fast and reproducible method is developed to obtain poly[(2-ethyl-2-oxazoline)-*co*-(ethylenimine)] (P(EtOx-*co*-EI)) copolymers *via* acid-catalyzed partial hydrolysis of poly(2-ethyl-2-oxazoline) (PEtOx). The hydrolysis kinetics were investigated by ^{1}H-NMR spectroscopy and size exclusion chromatography using hexafluoroisopropanol as eluent. It was found that the hydrolysis was greatly accelerated by increasing temperature from 100 ºC up to near-critical water (275 ºC) using microwave reactors. The optimal hydrolysis with regard to speed and control over the final copolymer structure was achieved at 180 ºC, since the polymer was found to degrade and decompose above this temperature. In addition, control over the desired degree of hydrolysis of PEtOx was obtained by selecting the appropriate HCl concentration. Summarizing, this work reports on defining optimal conditions to achieve tailored P(EtOx-*co*-EI) copolymers in a fast and reproducible way, utilizing high temperatures and controlled acidic conditions.

Introduction

Cationic ring opening polymerization (CROP) of 2-alkyl-2-oxazolines affords poly(2-alkyl-2--oxazoline)s (PAOx) with a narrow molecular weight distribution, tunable properties and excellent biocompatibility,[1] mostly documented for the water-soluble poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) (PEtOx), ascribed to their structural analogy with poly(peptide)s.[2] Telechelic polymers are readily obtained by selection of initiator (typically alkyl halides, (pluri)tosylates, (pluri)nosylates, etc.) and end-capping agent (a nucleophile),[3] while side-chains can be tuned by modification of the 2-substituent of the 2-oxazoline monomer.[4] Block copolymers can be obtained by sequential one-pot monomer addition, as a result of the living character of the CROP of 2-oxazolines. The use of microwave synthesizers has exerted a tremendous impact on the polymerization of 2-oxazolines by reducing the polymerization times to minutes or even seconds, thus allowing for high-throughput polymer synthesis and a systematic study of structure-property relationships.[5, 6] Therefore, PAOx have become a promising candidate for varying biomedical applications,[7-10] exhibiting higher synthetic versatility than the ubiquitous poly(ethylene glycol) (PEG).[11, 12]

The hydrolysis of PAOx renders linear polyethylenimine (L-PEI), constituting the main method to synthesize well defined L-PEI with interesting solubility properties due to its crystallinity and pH responsiveness. Moreover, L-PEI has been widely studied as a successful non-viral vector for gene delivery, outperforming the gold standard branched-PEI in terms of toxicity,[13-17] and in other high added-value biomedical applications.[18]

The partial hydrolysis of PAOx results in poly[(2-alkyl-2-oxazoline)-*co*-ethylenimine] (PAOx-*co*-PEI) copolymers that encompass both the interesting properties of PAOx and PEI while being less cytotoxic than L-PEI (see **Scheme 1**). These copolymers are responsive to external stimuli such as temperature and pH[19, 20] finding applications in aqueous self-assembly, micellar catalysis, or drug delivery.[21, 22] Indeed, the conjunction of PEI domains with stealth polymers such as PAOx or PEG has demonstrated to meet the DNA and RNA transfection efficiency of the commercial L-PEI, while reducing cytotoxicity and facilitating the introduction of targeting moieties for localized gene therapy.[23-27]

The introduction of secondary amines throughout the PAOx backbone in PAOx-*co*-PEI also offers a reactive handle for further modification by post-polymerization functionalization, further expanding the synthetic versatility and polymer architectures attainable.[28-31] For instance, PAOx graft copolymers can be readily synthesized by terminating living PAOx chains with the secondary amines present in PAOx-*co*-PEI copolymers.[32] These secondary amines can also be used for cross-linking resulting in pH-responsive hydrogels and nanogels for biomedical applications, as recently reported by Lecommandoux *et al.*[33]

As seen in the aforementioned applications, control over the

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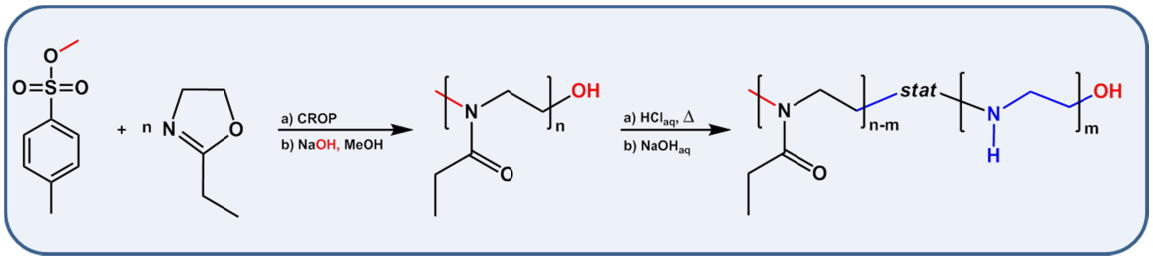

Scheme 1. General scheme for the cationic ring-opening polymerization of 2-ethyl-2-oxazoline initiated by methyl tosylate and terminated by –OH, followed by partial hydrolysis in HCl$_{(aq)}$.

hydrolysis degree is crucial to tailor the properties of the final PAOx-*co*-PEI materials. Since first reported by Saegusa over 40 years ago,[34] the synthesis of L-PEI *via* PAOx hydrolysis has been performed under strong acidic or alkaline conditions exhibiting pseudo-first order kinetics, with a direct correlation between acid or base concentration and hydrolysis rate. In addition, recent literature demonstrated the dependence of hydrolysis rate on the PAOx side chain,[19] giving rise to stimuli-responsive micelles,[20] and *quasi*-diblock P(EtOx-EI) copolymers by controlled partial hydrolysis.[35]

Acidic conditions were found to be preferred as they afford a faster and more controlled hydrolysis process by assuring total solubility of the formed PEI domains that are protonated at low pH.[36] Moreover, size exclusion chromatography (SEC) using hexafluoroisopropanol (HFIP) as eluent revealed degradation during basic hydrolysis.[20] Typically, the hydrolysis is performed under reflux conditions with concentrated HCl$_{(aq)}$ (usually 6 M), and the extent of hydrolysis controlled by the reaction time. However, under these conditions, small fluctuations in temperature and reaction time can result in large variations on the final degree of hydrolysis, thereby strongly impairing reproducibility, as we recently reported.[37]

Despite this previous research, there are no fast, accurate and reproducible methods available to obtain partial hydrolysis of poly(2-oxazoline)s. The main remaining difficulty being the accurate and reproducible control on the hydrolysis degree over the whole hydrolysis range, especially in short reaction times.[37] In the present research, we report on the effect of increasing temperature on the hydrolysis kinetics of PEtOx using only 1 M HCl concentration. Furthermore we studied the impact of high temperature on the structural integrity of the polymers, to determine the optimal conditions for controlled hydrolysis of PEtOx. In addition, variation of the acid concentration is demonstrated to be an effective way to accurately tailor the PEtOx degree of hydrolysis.

This article therefore describes a new robust methodology to obtain partially hydrolysed poly(2-oxazoline)s of reproducible composition in a fast manner, by lowering the acid concentration and increasing temperature.

Experimental

Materials and Instrumentation

2-Ethyl-2-oxazoline (99%) and methyl tosylate (98%) (Aldrich) were distilled over barium oxide (BaO, 97%) and stored under argon. Acetonitrile (CH_3CN, Acros Organics) was dried over activated molecular sieves (3Å). Poly(2-ethyl-2-oxazoline) 50 kDa (Aquazol® 50) and 200 kDa were purchased from Aldrich, HCl (37 (w/w) %, Aldrich) and NaOH (>97%), Acros Organics. CD_3OD (99.8% D), Euriso-top, France.

Deionized water was obtained from a Sartorius Arium 611 with a Sartopore 2 150 (0.45 + 0.2 µm pore size) cartridge filter (resistivity less than 18.2 MΩ cm).

SEC-DMA was performed on an Agilent 1260-series HPLC system equipped with a 1260 ISO-pump, a 1260 Diode Array Detector (DAD), a 1260 Refractive Index Detector (RID), and two PLgel 5 µm mixed-D columns in series, inside a 1260 Thermostated Column Compartment (TCC) at 50°C. The used eluent was DMA (>99.5%, Aldrich) containing 50 mM of LiCl at a flow rate of 0.593 mL/min. Molar masses and Đ values were calculated against PMMA standards from polymer labs.

SEC-HFIP was performed on an analogous Agilent 1260-series system, equipped with two PL HFIP gel columns (250 x 4.6 mm) in series. The eluent used was HFIP (Apollo scientific limited) containing 22 mM of sodium trifluoroacetate (NaTFAc) at a flow rate of 0.3 mL min^{-1}. Molar masses and Đ values were calculated against PMMA standards from Polymer Labs.

The samples corresponding to the PEtOx 3 kDa hydrolysis experiments with various HCl concentrations were measured in a system equipped with a Shimadzu LC-10AD pump, a waters 2414 refractive index detector (35 °C), a Spark Holland MIDAS injector, and a PSS PFG guard column followed by two PFG-linear-XL (7 µm, 8 x 300 mm) columns in series at 40 °C. Hexafluoroisopropanol (HFIP, Apollo Scientific Limited) with potassium trifluoroacetate (3 g L^{-1}) was used as eluent (flow rate of 0.8 mL min^{-1}), and the molar masses were calculated against polystyrene standards.

Proton NMR spectra were recorded in a Bruker Avance 300 MHz spectrometer at 25 ºC. The chemical shifts are given relative to

TMS.

The samples were dried in a freeze-drier (Thermo-electron Corporation) equipped with a vacuum pump (Pfeiffer) and a Heto drywinner cooling system.

Microwave Chemistry

Single-Mode Reactor. The synthesis of poly(2-ethyl-2-oxazoline), and the hydrolysis experiments were performed in a Monowave 300 microwave synthesizer, from Anton Paar GmbH, equipped with a MAS 24 auto sampler. Temperature was monitored with the built-in IR sensor. For the polymer synthesis, commercial caps were used. For the hydrolysis experiments, to ensure integrity of the microwave vials' septa at high temperatures and pressures under acidic conditions, custom septa were used. They comprised one 22 mm Ø, 2mm thick PTFE disk, and a 22 mm Ø, 1mm thick silicon/PTFE septum placed underneath (Bartelt GmbH).

Multi-Mode Reactor. The near critical water (NCW) experiments were carried out in a Multiwave 3000 multimode microwave reactor from Anton Paar GmbH. The instrument was fitted with two magnetrons, with continuous microwave output power from 0 to 1400 W. The cavity was fitted with an eight-vessel rotor, with 80 mL quartz glass vessels dedicated for reactions at high pressure (80 bar controlled pressure) and temperature (300 °C). Accurate temperature measurement was achieved by inserting a thermometer into one reference vessel. Additionally, the surface temperatures of all vessels could be monitored by IR. Pressure was monitored by a load-cell-type simultaneous hydraulic pressure sensing system for all vessels, with monitoring of the highest pressure level and pressure increase. The reactor's built-in electronics allowed reaction control in a temperature vs. time mode. After irradiation, the rotor was cooled to approximately 40 °C within 20 minutes.

General Procedure for the MW-NCW Experiments. A quartz vessel (80 ml) fitted with a Teflon-coated stirring bar was loaded with a PEtOx 200 kDa solution (0.48 M amide concentration) containing NaCl (0.03 M), for a total volume of 15 ml. The vessel was sealed and inserted into the 8-position rotor at position 1. Another 80 mL quartz vessel was fitted with an identical stirring bar and filled with NaCl solution (0.03 M, 15 mL). After sealing, this vessel was placed at position 5. Additionally, two sealed vessels containing only NaCl 0.03M solution were placed at positions 3 and 7 (as the rotor top plate contains the hydraulic system for simultaneous pressure sensing it is important to charge the rotor symmetrically; four fitted positions are necessary to achieve a flat position of the plate to guarantee accurate pressure measurement). After the vessels had been fixed by tightening the screws of the rotor top plate, the temperature probe was inserted into vessel 1. Finally, the rotor was closed with the protection hood and placed inside the cavity of the microwave reactor.

Kinetics investigations for the Hydrolysis of poly(2-ethyl-2-oxazoline) (PEtOx) 3 kDa

Synthesis of poly(2-ethyl-2-oxazoline) 3 kDa. PEtOx with a DP_n of 30 was synthesized via cationic ring-opening polymerization of EtOx. A 4 M solution of the monomer was prepared in acetonitrile together with methyl tosylate, resulting in a monomer to initiator ratio of 30. The solution was heated for 40 min. at 100 ºC under microwave irradiation, calculated to reach $\ln([M]_0/[M]_t) = 4$ (98% conversion),[31] cooled down to 40 ºC, and quenched by the addition of $NaOH/H_2O$ ensuring full deprotonation of the formed propionic acid making sure that it is not lost during solvent evaporation and can quantitatively be detected by NMR. The synthesized polymer was analyzed by SEC to determine the molar mass distribution and dispersity (Đ), and by ^{1}H NMR spectroscopy in $CDCl_3$ to ascertain near quantitative monomer conversion. The resulting homopolymer was dried under reduced pressure, redissolved in dichloromethane and purified by precipitation in cold diethyl ether.

^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.80 – 3.28 (4H, -C*H*$_2$-C*H*$_2$-N-), 3.1 – 2.8 (3H^{ini}, C*H*$_3$-NCOCH$_2$CH$_3$), 2.45 – 2.13 (2H; -NCOC*H*$_2$-CH$_3$), 1.15 – 0.95 (3H; -NCOCH$_2$C*H*$_3$).

$PEtOx_{30}$: $M_{n,\ ^1H\text{-}NMR} = 2900$ Da. $M_{n,\ DMA\text{-}SEC} = 6000$ Da; $Đ_{DMA\text{-}SEC} = 1.07$. $M_{n,\ HFIP\text{-}SEC} = 3300$ Da; $Đ_{HFIP\text{-}SEC} = 1.55$.

Acidic Hydrolysis of poly(2-ethyl-2-oxazoline) 3kDa. The hydrolysis kinetics were performed either in 4 mL pressure tubes (Ace glass Inc.) in an oil bath, or in a monomode microwave synthesizer. The desired total volume in each vial (2-5 ml microwave vial) was 3 mL with a concentration of 1M $HCl_{(aq)}$. Therefore 0.25 mL of a 36 wt.% (11.96 M $HCl_{(aq)}$) solution was mixed with 2.75 ml of PEtOx 3 kDa stock solution (amide concentration = [A] = 0.48 M). The vials were heated for different times at temperatures ranging from 120 ºC to 220 ºC. Upon completion of the desired reaction time, the obtained reaction mixture was cooled down by compressed air and made basic with 1 mL of a 4 M $NaOH_{(aq)}$ solution to a pH of 8-9. Subsequently, the samples were freeze dried for HFIP-SEC and ^{1}H-NMR spectroscopy.

To determine the hydrolysis kinetics at 180 ºC with different HCl concentrations, a stock solution of PEtOx 3kDa (0.53 M amide concentration) was prepared. A 18 mL portion was taken and the necessary amounts of 36 wt.% $HCl_{(aq)}$ were added, completing the volume to 20mL with milli-Q water to obtain the desired $HCl_{(aq)}$ concentration and 0.48 M amide concentration. The work up and analysis were performed analogously as for the hydrolysis with 1 M HCl concentration (*vide supra*).

^{1}H-NMR characterization to calculate the Hydrolysis Conversion. The conversion is calculated from ^{1}H-NMR spectra in deuterated methanol, using the signals of the hydrolysis products. All the signals described for PEtOx are present, together with the signals correspondent to the respective hydrolysis products (see **Scheme S1**).

P[(EtOx$_{30-x}$)-*co*-(EI)$_x$]: ^{1}H NMR (300 MHz, CD_3OD, δ): $PEtOx_{30}$ + hydrolysis products: 3.80 – 3.28 (4H, -C*H*$_2$-C*H*$_2$-N-), 3.00 – 2.65 (4H, -NH-C*H*$_2$-C*H*$_2$-), 2.45 – 2.13 (2H; -NCOC*H*$_2$-CH$_3$), 2.20 – 2.00 (CH$_3$C*H*$_2$COOH), 1.20 – 0.85 (3H, -NCOCH$_2$C*H*$_3$, 3H, C*H*$_3$CH$_2$COOH).

The calculation method used for determining the composition is based on the integral values (I), as displayed in the following equations:

$$\%\ \text{Conv. PEtOx} = \frac{\mathrm{I}[PEI\ backbone]}{\mathrm{I}[PEI\ backbone] + \mathrm{I}[PEtOx\ backbone]} \times 100$$

$$\%\ \text{Conv. PEtOx} = \frac{\mathrm{I}[PEI\ backbone]}{2(\mathrm{I}[EtOx\ CH_2]) + \mathrm{I}[PEI\ backbone]} \times 100$$

The reported degree of hydrolysis was calculated by the average of the results obtained *via* these equations. The conversion values calculated from these equations were found to differ by less than 5%.

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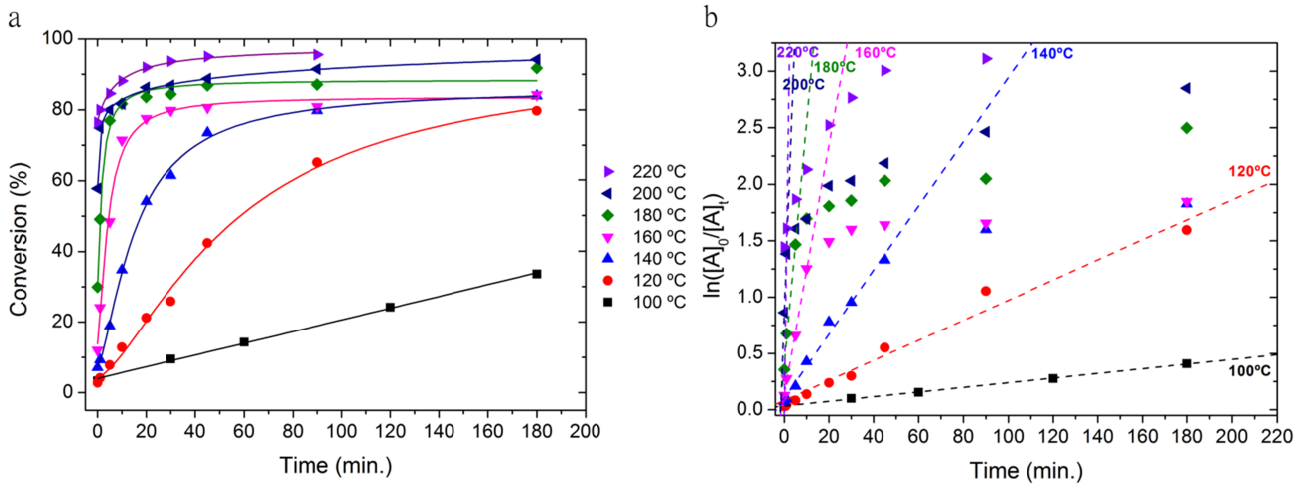

Figure 1. a) Conversion versus time plot for the hydrolysis of PEtOx 3 kDa at temperatures ranging from reflux conditions to 220 °C. [HCl] = 1.0 M. Increasing temperature greatly accelerates the hydrolysis rate. **b)** Corresponding first order kinetic plot. The deviation from linearity beyond 80% conversion can be ascribed to the protonation of the formed ethylenimine units and the consequent decrease of free protons available to catalyze the hydrolysis.

Results and discussion

Accelerating PEtOx hydrolysis: Hydrolysis Kinetics of Poly(2-ethyl-2-oxazoline) at elevated temperatures

The use of a microwave synthesizer allows for performing PEtOx hydrolysis at higher temperatures, and can reduce reaction times to an order of minutes. Recently, the full hydrolysis of PEtOx to obtain pure L-PEI was optimized by using high acid concentration (3M) and a temperature slightly beyond reflux conditions (130 ºC), by means of a microwave synthesizer.[38] In the current research, we aimed to control fast partial hydrolysis of PEtOx. Therefore, milder acidic conditions, *i.e.* 1 M HCl concentration, and higher temperatures were utilized. In order to ascertain the maximum temperature at which the hydrolysis of PEtOx can be performed, without compromising the polymer integrity, PEtOx solutions were heated for different times at temperatures ranging from 100 ºC to 220 ºC under microwave irradiation. The obtained P(EtOx-*co*-EI) copolymers were basidified with $NaOH_{(aq)}$ and the conversion from PEtOx to PEI was calculated based on ^{1}H-nuclear magnetic resonance (^{1}H-NMR) spectroscopy (see Experimental Section for details). The structural integrity of the resulting copolymers was assessed by SEC with HFIP as eluent.

The temperature was found to strongly impact the hydrolysis kinetics of PEtOx, sharply accelerating the hydrolysis rate even when heating only 20 ºC above reflux conditions (see **Figure 1**). Increasing reaction temperature thus constitutes an effective way to hydrolyze PEtOx under much milder acidic conditions than previously reported.

The hydrolysis of PEtOx has been shown to follow a pseudo-first order kinetics mechanism, where hydronium ions act as a catalyst and thus their concentration remains *quasi* constant during the hydrolysis.[19] To confirm our previous finding that the hydrolysis rate is independent of molar mass, we utilized PEtOx with an M_n of 3 kDa synthesized in house, and a commercial PEtOx with an M_n of 50 kDa, *vide infra*. However, in the current research the kinetic plot deviates from linearity levelling-off after *ca.* 80% conversion, as shown in **Figure 1b**. This observation can be ascribed to the protonation of the ethylene imine units formed during the hydrolysis, reducing the availability of hydronium ions to catalyze the reaction, and consequently decreasing the reaction rate (see **Equation** 1).

$$\text{Conversion} \propto \ln\left(\frac{[A]_0}{[A]_t}\right) = k_h[H^+]t$$

Equation 1.: Integrated pseudo-first order rate law. Initial amide concentration, $[A]_0$ = 0.48M. k_h: hydrolysis rate constant (L mol^{-1} s^{-1}), $[H^+]$ = 1M. The conversion increases logarithmically with the hydronium concentration.

This hydronium depletion upon PEtOx hydrolysis has not been previously observed as a deviation from linear first order kinetics since former reports made use of a large HCl excess, thereby masking the effect of amine protonation. In the present study, the HCl concentration (1 M) was just above twice the PEtOx amide concentration (0.48 M) and thus, the loss of hydronium ions by protonation of the amines has a significant effect on the hydrolysis kinetics.

To quantify these experimental results, the initial linear part of the kinetic plots was fitted and the apparent hydrolysis rate constant (k_h^{app}) calculated for each temperature. **Figure 2** shows the corresponding Arrhenius plot from which a value of 76 ± 1 kJ mol^{-1} for the activation energy (E_a) was calculated. These k_h^{app} are in good agreement with previously reported values[‡] for hydrolysis studies performed at lower temperatures (from 57 to 100 ºC) and a high acid concentration of 6 M.[37]

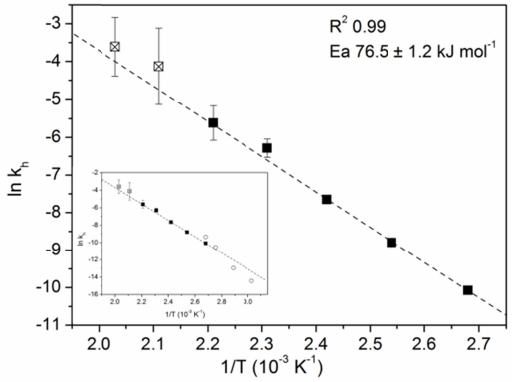

Figure 2. Arrhenius plot for the acid hydrolysis of PEtOx 3 kDa (1 M $HCl_{(aq)}$) at temperatures ranging from 100 °C to 220 °C. The data points corresponding to 200 °C and 220 °C (( ) were not considered for the calculation of the E_a, as the polymer degrades at these temperatures (*vide infra*). The inset includes previously reported data[‡] (0) obtained at temperatures from 57 °C to 100 °C with [HCl] = 5.8 M, and is in good agreement with the current results.[37]

SEC in hexafluoroisopropanol, which was recently found to be an excellent eluent for L-PEI,[19] was used to investigate the influence of temperature on the structural integrity of the obtained P(EtOx-*co*-EI) copolymers. PEI has a larger hydrodynamic volume than PEtOx and consequently the retention time and molecular weight calculated for P(EtOx-*co*-EI) copolymers increases with the degree of hydrolysis. **Figure 3** displays the evolution of the measured number average molecular weight (M_n) with the hydrolysis conversion at different temperatures. It should be noted that the absolute M_n values at 120, 160, and 200 ºC are higher than those obtained by hydrolysis at 140, 180 and 220 ºC, which can be ascribed to a change in the SEC system (possible variation in trifluoroacetate salt concentration) and recalibration in between these two series of experiments. Nonetheless, the expected trend towards higher Mn values with increasing conversion is observed; however, at temperatures of 200 ºC and higher, the M_n values abruptly drop as a consequence of polymer backbone degradation (see Supporting Information for SEC traces). At these temperatures and pressures (close to 30 bar), the energy applied to the polymer appears to be sufficiently high for the dissociation of its main chain (-CH_2-CH_2-NH-)$_n$ bonds. These results clearly indicate that 180 ºC is the maximum temperature at which the hydrolysis can be performed using 1 M concentration HCl without affecting the polymer integrity. By consequence, PEtOx hydrolysis cannot be further accelerated under these conditions.

To understand the influence of acid on polymer degradation, and to elucidate whether the hydrolysis could be performed under acid-free conditions, the hydrolysis of PEtOx was also investigated in near-critical water using a multimode microwave reactor.[39, 40] The polymer was dissolved in an aqueous NaCl 0.03 M solution, and the vial was heated to 270 ºC (IR sensor) for 60 minutes, with the pressure reaching 80 bar. The addition of NaCl as electrolyte is necessary, as the drop in dielectric constant of near-critical water turns it nearly transparent to microwave radiation. Under these acid-free conditions, PEtOx was readily hydrolyzed but it also degraded over time, obtaining nearly complete decomposition of the polymer after 1h of reaction, and thus indicating acid-free hydrolysis of PEtOx to be impossible.

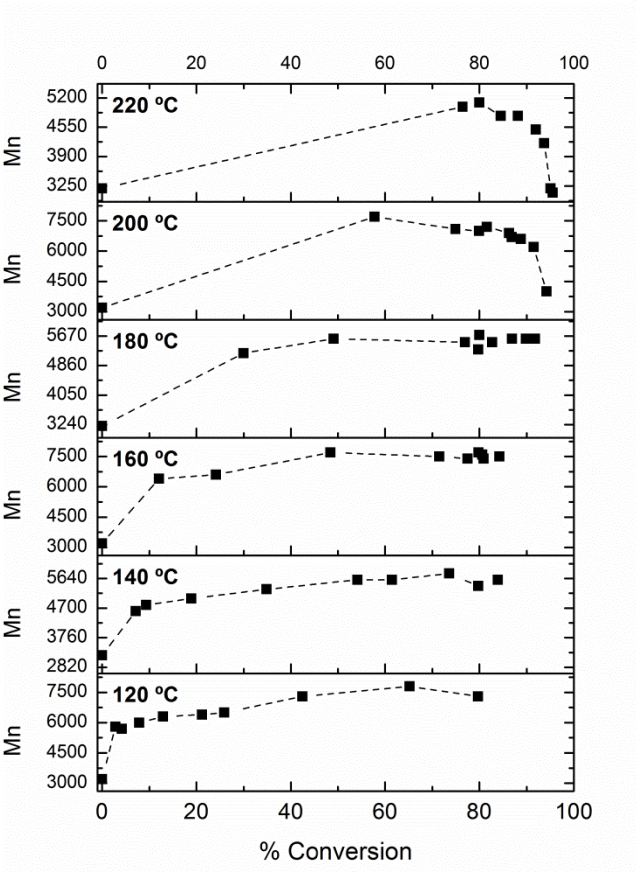

Figure 3. Number average molecular weight (M_n) of the obtained PEtOx-*co*-PEI copolymers plotted against the degree of hydrolysis, measured by HFIP-SEC. Dashed lines are added to guide the eye: The M_n increases with the conversion, as PEI units result in an increase on the hydrodynamic volume of the copolymer. However, at temperatures higher than 180 °C, a sharp decrease in the M_n values is observed, indicating degradation of the polymer backbone into oligomers.

To study the influence of PEtOx molecular weight, analogous hydrolysis experiments were performed using commercial PEtOx with a M_n of 50 kDa (Aquazol 50). Hydrolysis experiments of this larger PEtOx yielded comparable results as obtained for PEtOx 3 kDa, which was expected from previous research.[19] The results corresponding to PEtOx 50 kDa are included in the Supporting Information.

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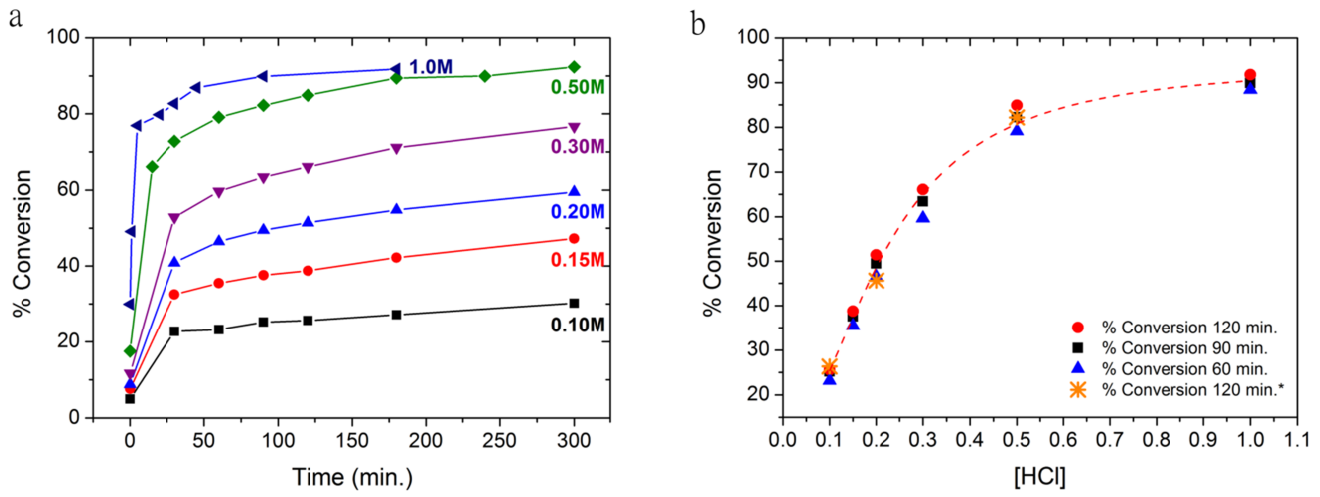

Figure 4. a) Conversion versus time plot for the hydrolysis of PEtOx 3 kDa (amide concentration = [A] = 0.48M) at 180 °C under various HCl concentrations ranging from 0.10 M to 1.0 M. The degree of hydrolysis levels off at defined values at each acid concentration, therefore allowing good control over the degree of hydrolysis. **b)** Conversion plotted against HCl concentration. * 〃 Reactions performed in closed pressure tubes and conventional heating instead of microwave heating. The robustness of the partial hydrolysis is demonstrated by the small impact that variations of 30 minutes of reaction time have on the degree of hydrolysis.

Controlling PEtOx hydrolysis by variation of acid concentration

Once the optimal temperature was found to be 180 ºC, we focused our attention on gathering precise control over the degree of hydrolysis. Assuming that the aforementioned hydronium depletion upon hydrolysis accounts for the flattening of the first order kinetic plots, it was hypothesized that sub-stoichiometric amounts of HCl would prevent hydrolysis to proceed beyond a specific degree of conversion. To explore this hypothesis, a series of HCl concentrations ranging from 0.1 M to 1M was used to partially hydrolyze PEtOx at 180 ºC. A fast hydrolysis was found in all cases, up to a plateau that correlates with the acid concentration used (see **Figure 4a**). After an initial fast hydrolysis, the degree of hydrolysis stabilized after 30 minutes, only slowly increasing thereafter. The robustness of this acid-concentration controlled hydrolysis is demonstrated in **Figure 4b**: when the hydrolysis was performed for 90 minutes, a variation of ± 30 minutes in reaction time only had a minor impact on the hydrolysis conversion, demonstrating accurate control over the degree of hydrolysis in a reproducible manner. The hydrolysis was also performed in closed pressure tubes with conventional heating, to demonstrate the applicability of the method beyond microwave chemistry. It is evident from Figure 4b that very similar hydrolysis degrees are obtained with conventional heating further demonstrating the robustness of this novel method to control the hydrolysis degree by the acid concentration, independent of the heating method.

During the PEtOx hydrolysis, the produced ethylene imine (EI) units are protonated, since the pH of the reaction medium is below the pKa of L-PEI (pKa ≈ 7.2-7.9).[41] Consequently, every newly formed EI unit drains a hydronium ion from the acidic solution. This translates to a pH neutralization of the solution at the point where the concentration of EI groups equals the initial acid concentration, preventing further hydrolysis. The released propionic acid units only have a minor influence as they are insufficiently acidic to hydrolyze PEtOx. **Figure 5** shows the relationship between EI concentration present after 90 min. reaction time and the initial HCl concentration used. A direct relationship between $[HCl]_0$ and $[EI]_{90min}$ is observed under sub-stoichiometric HCl concentrations ($[HCl]_0$ < [A] = amide concentration = 0.48 M), demonstrating that controlling the initial HCl concentration constitutes an effective method to tailor and accurately control the degree of hydrolysis of the resulting P(EtOx-*co*-EI) copolymer.

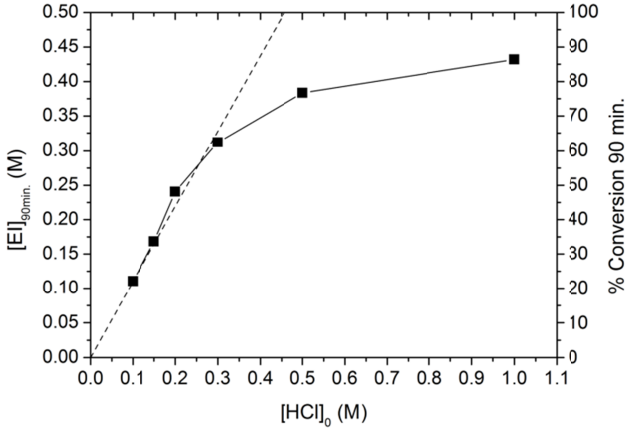

Figure 5. Concentration of ethylene-imine units ($[EI]_{90min}$) formed upon hydrolysis versus HCl concentration, showing a close relationship between both. The right axis indicates the corresponding % conversion of amide side-chains into EI. The dotted line guides the eye through the linear region of the curve, where sub-stoichiometric amounts of HCl are used. Data from the hydrolysis of PEtOx 3 kDa, amide concentration = [A] = 0.48 M, T = 180 °C.

Conclusions

The hydrolysis kinetics of PEtOx 3 kDa and 50 kDa in the presence of 1 M HCl at different temperatures are reported. Increasing the reaction temperature is demonstrated to be an effective way to accelerate the hydrolysis rate, even when comparatively low acid concentrations were used. The maximum acceleration can be achieved at 180 ºC, as the polymer degrades into oligomers at higher temperatures. Acid-free, near-critical water conditions (275 ºC, 80 bar) also led to polymer decomposition into oligomers after only 60 min., indicating the inability to perform PEtOx hydrolysis in pure water.

In addition, the evolution of the hydrolysis conversion over time was found to abruptly level off at defined values dependent on the acid concentration. When using sub-stoichiometric concentrations of HCl, the ethylenimine units generated upon hydrolysis are protonated, capturing hydronium ions from the reaction medium and consequently halting the hydrolysis reaction. The proposed method provides accurate control on the final P(EtOx-*co*-EI) composition by judiciously selecting and controlling the acid concentration, while reducing reaction times and rationalizing the use of acid. Despite the ease of performing autoclave reactions in modern microwave reactors, this method is not limited to such specialized equipment and can also perfectly be utilized in closed pressure reactors, therefore finding applicability beyond microwave chemistry.

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Notes and references

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† Electronic Supplementary Information (ESI) available: NMR data, SEC traces, hydrolysis kinetics results for PEtOx 50 kDa, and results for the acid-free near-critical water hydrolysis of PEtOx can be found in the electronic Supporting Information. See DOI: 10.1039/b000000x/

‡ It should be noted that these previously reported k_h values[37] were found to be 10^3 times lower than reported, likely due to a typographic error.

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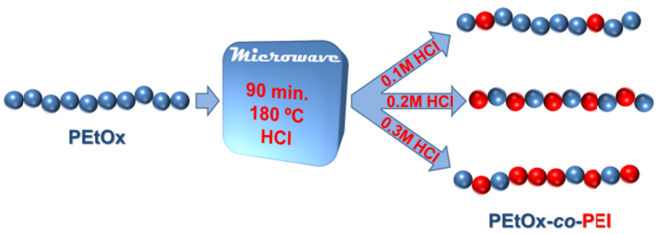
GRAPHICAL ABSTRACT



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This work reports on defining optimal conditions to achieve tailored P(EtOx-*co*-EI) copolymers in a fast and reproducible way, utilizing high temperatures and controlled acidic conditions.