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Linear Poly(ethylene imine)s by Acidic Hydrolysis of Poly(2-oxazoline)s: Kinetic Screening, Thermal Properties, and Temperature-Induced Solubility Transitions

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ABSTRACT: The kinetics for the acidic hydrolysis of two water-soluble poly(2-oxazoline)s with methyl and ethyl substituents were investigated. It could be observed that poly(2-ethyl-2-oxazoline) (PEtOx) and poly(2-methyl-2-oxazoline) (PMeOx) are hydrolyzed following a linear relation with time. Various polymer lengths and concentrations were investigated, revealing that both parameters had no influence on the hydrolysis kinetics. Comparison between PEtOx and PMeOx revealed that the smaller PMeOx side group could be removed faster. Furthermore, a series of linear poly(ethylene imine) (PEI) were synthesized with varying M/I ratio from 5 to 200 to elucidate structure−property relations. Thermal measurements indicated that the T_g of PMeOx was increasing up to a M/I ratio of 100. PEI, on the other hand, is crystalline and exhibits a melting temperature which shows a similar increase with M/I ratio as observed for the T_g of PMeOx. Solubility measurements in water and water/ethanol mixtures indicated a solubility transition to a soluble state at elevated temperatures. The solubility transition was observed to fit with the melting temperature, indicating that the PEI crystals need to melt in solution before solubilizing. Moreover, when adding ethanol to the water solution, the formed crystals are less stable due to the improved solubility of PEI in ethanol, causing a decrease in cloud point temperature. Upon cooling, PEI crystallizes at much lower temperatures compared to dissolution, causing a large hysteresis presumably due to extensive intermolecular hydrogen bonding, which also plays a role in the formation of the hydrate crystals.

Introduction

Poly(ethylene imine) (PEI) is best known as a highly branched polymer containing primary, secondary, and tertiary amino functions, which can be obtained by the cationic ring-opening polymerization of ethylene imine. The branching is caused by the secondary amine groups in the polymer chain which have similar basicity as the monomer. Therefore, chain transfer to polymer takes place, resulting in branching.[1,2] It is relatively easy to attach a variety of organic groups to PEI and to introduce desired functionalities.[3,4] Efforts have been made to prepare linear PEI, which has different properties compared to the branched PEI.

Tomalia et al.[5] reported in 1966 one of the first manuscripts on the synthesis of poly(2-oxazoline)s. To prove the polyimide structure, the authors hydrolyzed poly(2-methyl-2-oxazoline) to PEI by using sulfuric acid and proved the formation of acetic acid. The living cationic ring-opening polymerization of 2-oxazolines is a common method for the synthesis of well-defined polymers with narrow molar mass distribution.[6−16] Therefore, the hydrolysis of poly(2-oxazoline)s represents a promising route toward the preparation of well-defined linear PEI, allowing the determination of structure−property relationships.

The first detailed characterization of linear PEI was published by Saegusa et al.[17] starting from the living cationic ring-opening polymerization of 2-oxazoline and subsequent hydrolysis of the poly(2-oxazoline) under alkaline conditions. A crystalline product was obtained with a melting temperature of 58.5 °C and a glass transition temperature of −23.5 °C. Since then, poly(2-oxazoline)s are often used as precursor for linear PEI upon hydrolysis under alkaline[18] or acidic[19,20] conditions. In addition, Wang et al. reported that PEtOx could also be partially hydrolyzed via enzymatic hydrolysis.[21]

PEI is used in various applications, for example in the textile industry[22,23] where PEI is utilized for a pretreatment of fibers for a better adsorption or desorption of dyes. Other applications can be found in the treatment of paper.[24] Furthermore, PEI is a commonly used polymer vector for DNA and RNA delivery into cells. PEI has a very high charge density because every third atom (nitrogen atom) can be protonated, making PEI capable of condensing plasmid DNA and RNA into stable complexes via electrostatic interactions.[20,25,26] Linear PEI is also known to exhibit interesting solubility properties since it is only soluble in water at elevated temperatures and insoluble in water at room temperature, as reported earlier by Saegusa.[17] This specific behavior is ascribed to the phase separation occurring upon the

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crystallization of PEI. Linear PEI is known to be highly crystalline due to the unsubstituted, flexible polymer chains which are insoluble at room temperature and melt in solution to a hydrated state at elevated temperatures.[27]

In this study we investigated the kinetics for the acidic hydrolysis of two water-soluble poly(2-oxazolines) with methyl and ethyl substituents. Various polymer lengths and concentrations were investigated while keeping the acid concentration constant. On the basis of the obtained kinetics, a series of linear PEIs with varying chain lengths were synthesized utilizing poly(2-methyl-2-oxazoline) as starting material. All obtained PEI samples were analyzed for their thermal and solubility properties in water and water/ethanol mixtures. To the best of our knowledge, this represents the first detailed study on the thermal and solubility properties of linear PEI with varying chain lengths.

Experimental Section

Instrumentation. All chemicals were purchased from Aldrich. The 2-oxazoline monomers and methyl tosylate were distilled over barium oxide (BaO) and stored under argon. Acetonitrile (CH_3CN, Aldrich) was dried over molecular sieves (3 Å). Polymerizations were carried out in a single-mode microwave reactor Emrys Liberator (Biotage) with capped reaction vials. These vials were heated to 105 °C, allowed to cool to room temperature, and filled with argon before use. All microwave polymerizations were performed under temperature control (IR sensor).

^{1}H NMR spectra were recorded on a Varian AM-400 spectrometer and analyzed in deuterated methanol. Chemical shifts are given in part per million with respect to tetramethylsilane or residual solvent signals. Size exclusion chromatography (SEC) was measured on a system equipped with a Shimadzu LC-10AD pump, a Waters 2414 refractive index detector (35 °C), a Spark Holland MIDAS injector, a PSS PFG guard column followed by two PFG-linear-XL (7 μm, 8 × 300 mm) columns in series at 40 °C. Hexafluoroisopropanol (HFIP, Apollo Scientific Limited) with potassium trifluoroacetate (3 g/L) was used as eluent (flow rate of 0.8 mL min^{-1}) since this was found to be the only suitable eluent for the analysis of PEI. The molar masses were calculated against polystyrene standards. MALDI-TOF MS measurements were performed with an Ultraflex III TOF/TOF (Bruker Daltonics, Bremen, Germany) mass spectrometer equipped with a Nd:YAG laser and a collision cell. The spectra were measured in reflector mode. The instrument was calibrated prior to each measurement with an external PMMA standard from PSS Polymer Standards Services GmbH (Mainz, Germany). MS data were processed using the software PolyTools 1.0 (Bruker Daltonics). For the sample preparation, PEI (10 mg/mL) in methanol, 2,5-dihydroxybenzoic acid in methanol (20 mg/mL), and no doping salt were used. The dried-droplet spotting technique[28−30] (matrix and analyte previously mixed together) was applied. For each sample 1 μL of the mixture was spotted onto a target plate.

Thermal transitions were determined on a DSC Q100 from TA Instruments. Sample weights were in the range of 3−10 mg. The samples were measured in hermetic aluminum cups with a heating and cooling rate of 20 K min^{-1} for both the glass transition temperatures and the melting points (two measurements per sample after an initial first heating run that was not considered for the subsequent calculations).

The cloud points of the polymer solutions were measured by heating the polymer (5.0 ± 0.2 mg) in a solvent mixture of ethanol (Biosolve) and deionized water (Laborpure, Behr Labor Technik) (1.0 mL). The investigated temperature range was 1−100 °C with heating and cooling ramps of 1 °C min^{-1}. During these controlled heating and cooling cycles (two cycles per sample), the transmission through the solutions was monitored with red light in a Crystal 16 from Avantium Technologies.[31] Four blocks of four parallel temperature controlled sample holders are connected to a Julabo FP40 cryostat, allowing 16 simultaneous measurements. All vials were visually inspected after the heating program to facilitate the interpretation of the observed transmission profiles. The presented cloud point temperatures correspond to the dissolution temperature at 50% transmittance from the second heating run.

Kinetic Investigations of the Acidic Hydrolysis of Poly(2-ethyl-2-oxazoline). Poly(2-ethyl-2-oxazoline)s (PEtOx) with varying monomer to initiator (M/I) ratio (M/I 20, 50, 100, and 150) were synthesized according to procedures described earlier.[32,33] All polymers were purified by precipitation into diethyl ether and subsequent drying under reduced pressure at 40 °C. For the kinetic screening, a stock solution with 16.8 wt % HCl in water was prepared. Separate microwave vials were used for each reaction which contained 96 ± 1.0 mg of PEtOx and 2 mL of acid stock solution, corresponding to a constant amide concentration of 0.48 M ($[PEtOx_{20}] = 24$ mM; $[PEtOx_{50}] = 10$ mM; $[PEtOx_{100}] = 5.0$ mM; $[PEtOx_{150}] = 3.3$ mM). Each vial was heated in the microwave synthesizer to 100 °C for different reaction times. After the desired reaction time, the obtained mixture was cooled by compressed nitrogen. The resulting mixtures were neutralized with 2.5 M NaOH solution to a pH value >8, and the remaining solvent was removed under reduced pressure. Subsequently, a small amount of deuterated methanol was added to the dried polymer, and ^{1}H NMR spectroscopy was used to determine the conversion of the poly(2-oxazoline) to PEI. To determine the conversion from PEtOx to PEI, the signals from the released propionic acid ($-CH_2$ at 2.1 ppm) and the signals from the remaining CH_2 group in the side chain of PEtOx ($-CH_2$ at 2.4 ppm) were used.

Kinetic Investigations of the Acidic Hydrolysis of Poly(2-methyl-2-oxazoline). A similar procedure was followed for the synthesis and hydrolysis of poly(2-methyl-2-oxazoline) (PMeOx) with a M/I ratio of 100. The conversion of PMeOx to PEI was determined utilizing the CH_3 group of the formed acetic acid (CH_3 1.9 ppm) and the remaining CH_3 groups of the PMeOx side chains (CH_3 2.1 ppm) in the ^{1}H NMR spectra.

The amount of $PMeOx_{100}$ was varied from 48 ± 1.0 to 41 ± 1.0 and 20 ± 1.0 mg/mL, corresponding to $[PMeOx_{100}] = 5.5$, 4.8, and 2.4 mM, respectively.

Synthesis of PMeOx and Subsequent Hydrolysis to Linear Poly(ethylene imine). The polymerization mixtures for the microwave-assisted synthesis of a series of PMeOx homopolymers were prepared manually. In each vial 2-methyl-2-oxazoline (MeOx), methyl tosylate, and acetonitrile were added, resulting in a total monomer concentration of 4 M and a varying monomer to initiator ratio between 5 and 200. The microwave vials were subsequently heated to 140 °C to reach full conversion. The polymerization mixtures were quenched after microwave heating by the automated addition of 50 μL of water. Even though the DP of the polymers cannot be easily determined by SEC or ^{1}H NMR spectroscopy, previous MALDI-TOF MS studies revealed that the DP of the polymers is very close to the aimed M/I ratio,[32] which is also assumed in the current study.

The resulting homopolymers were dried under reduced pressure at 40 °C, and further purification was performed by precipitation into diethyl ether. All synthesized polymers were subsequently analyzed by SEC to reveal the molar mass distribution and the polydispersity index. Further characterization was performed by DSC and solubility measurements to determine selected polymer properties. Subsequently, linear PEI was synthesized by the acidic hydrolysis of the PMeOx homopolymers. PMeOx was added to a 16.8 wt % HCl solution in water with a concentration of 48 g/L. The hydrolysis reaction was performed overnight under heating to reflux to obtain 100% conversion. After cooling the reaction mixture, the acidic solution was removed under reduced pressure. Deionized water was added, and the solution was neutralized with 2.5 M NaOH solution to a pH value >8. The formed precipitate was filtered off and recrystallized from deionized water. The product was

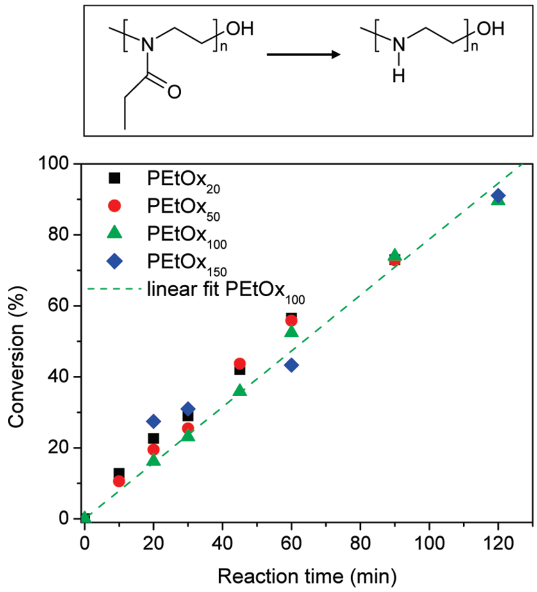

Figure 1. Top: schematic representation of the polymer structures. Bottom: conversion vs time plot for the acidic hydrolysis of PEtOx with varying chain length and constant amide concentration of 0.48 M.

finally obtained by freeze-drying to remove all remaining water. The obtained series of linear PEI were all characterized by ^{1}H NMR spectroscopy to prove complete hydrolysis and were analyzed by SEC to determine the molar mass distribution and the polydispersity index. Further characterization was performed by DSC and solubility measurements to compare selected polymer properties with the PMeOx homopolymers.

Results and Discussion

It is well-known that poly(2-oxazoline)s can be used to prepare linear poly(ethylene imine) (PEI) via alkaline[18] or acidic[19] hydrolysis. Here we investigate the kinetics of the acidic hydrolysis of two hydrophilic polymers, namely poly(2-ethyl-2-oxazoline) (PEtOx) and poly(2-methyl-2-oxazoline) (PMeOx), by varying the length of the polymer and the polymer concentration. Furthermore, a series of linear PEI were synthesized via the acidic hydrolysis of PMeOx with varying M/I ratios from 5 to 200. The thermal and solubility properties of these linear PEIs were compared to the starting PMeOx, and structure–property relationships were determined.

Kinetic Investigations of the Acidic Hydrolysis. The kinetics of acidic hydrolysis of two water-soluble poly(2-oxazoline)s, namely PEtOx and PMeOx, were investigated. First, the acidic cleavage of PEtOx with varying M/I ratios was performed using a constant amide concentration of 0.48 M in 16.8 wt % HCl in water. After heating separate reaction vessels for a predefined time to 100 °C, the conversion from PEtOx to PEI was determined by ^{1}H NMR spectroscopy. The results are depicted in Figure 1.

As can be observed, the hydrolysis to PEI is linearly correlated with the reaction time, and after about 120 min a conversion of 90% is obtained. A closer examination of the kinetic plots revealed a slightly slower reaction above 60% conversion, which is most evident for PEtOx$_{20}$ and PEtOx$_{50}$, due to the decreased solubility of the formed PEI in the acidic solution as demonstrated by partial precipitation. Furthermore, Figure 1 demonstrates that the length of the polymer has no significant influence on the hydrolysis rate, which might be expected since the concentration of amide groups is constant. Nonetheless, the absence of chain length dependence indicates that there are no other significant effects and that the side groups of PEtOx are all equally accessible independent of the length of the polymer. The acidic cleavage of water-soluble poly(2-oxazoline)s at 100 °C was reported to require a 1:1 stoichiometry of acid to amide,[34] which is important since it provides the possibility to control the partial cleavage offering desired properties for specific applications. In this study, however, we investigated the kinetic cleavage with a large excess of acid, and therefore, a conversion of 100% should be easily reachable. Furthermore, it was reported that acidic hydrolysis becomes slower if a conversion of ~70% is reached,[34] which is in agreement with the results depicted in Figure 1. In a next step, a comparison was made between the acidic hydrolysis of PEtOx and PMeOx, which are both hydrophilic and water-soluble. For comparison, PMeOx was investigated with a similar M/I ratio of 100, and the reaction conditions such as temperature and (acid) concentration were kept constant. The results are depicted in Figure 2.

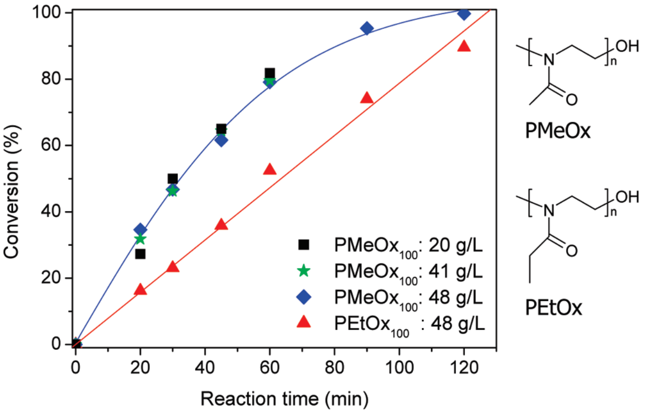

Figure 2. Conversion vs time plot for the acidic hydrolysis of PMeOx and PEtOx with varying concentration (PMeOx$_{100}$: 20 g/L = 2.4 mM, 41 g/L = 4.8 mM, and 48 g/L = 5.5 mM; PEtOx$_{100}$: 49 g/L = 4.8 mM).

Comparison of the hydrolysis of PMeOx and PEtOx reveals a faster reaction for PMeOx, which is also in agreement with the known amide kinetics: the extra carbon atom in the PEtOx side chain makes the tetrahedral intermediate more crowded, and therefore PMeOx is more easily cleaved. Furthermore, PMeOx is known to be more hydrophilic in comparison to PEtOx,[35,36] which also contributes to better hydration and, thus, faster cleavage. Additionally, the observed kinetics for PMeOx revealed an almost linear behavior up to a conversion of ~80% (Figure 2) while a small deviation from linearity was already observed around ~60% conversion for PEtOx. The higher hydrophilicity of PMeOx renders the partially hydrolyzed products better soluble. As a result, precipitation and associated deceleration of the hydrolysis occur at a higher conversion. Moreover, the effect of polymer concentration was investigated for PMeOx$_{100}$ by evaluating the hydrolysis kinetics for two additional polymer concentrations, namely 41 g/L ([PMeOx$_{100}$] = 4.8 mM) representing the same molar concentration as for PEtOx$_{100}$ as well as a lower polymer concentration of 20 g/L ([PMeOx$_{100}$] = 2.4 mM), while keeping the hydrochloric acid concentration constant at 16.8 wt % HCl in water. The results indicate that there is no influence of the polymer concentration on the speed of the acidic cleavage (Figure 2). This can be explained by the mechanism of the monomeric amide cleavage as documented in organic chemistry textbooks. At first, the acid–base reaction takes place, and the amide is activated by protonation of the amide carbonyl, making the carbon atom more electrophilic. The next step is known as the rate-determining step and comprises the formation of a tetrahedral intermediate by the attack of the oxygen from water on the electrophilic carbon atom. Finally, the side group is removed by a number of fast non-rate-determining steps. Because of the large excess of both hydrochloric acid and water, the effect of polymer concentration on the hydrolysis rate is negligible.

Synthesis of a Series of Linear Poly(ethylene imine)s with Varying M/I Ratios. A series of linear PEI with varying length were synthesized by acidic hydrolysis of PMeOx. These PEIs were investigated to study selected structure−property relationships in comparison to the starting PMeOx. The series of PMeOx polymers were synthesized with varying M/I ratio between 5 and 200 as reported previously.[32] All obtained polymers were characterized by SEC to determine the molar mass and the polydispersity index, which demonstrated that well-defined polymers were obtained with polydispersity indices mostly below 1.25 (Table 1). Only the PMeOx with M/I ratios of 150 and 200 have slightly higher polydispersity indices (1.41 and 1.40, respectively), which can be ascribed to chain transfer reactions and subsequent chain coupling.[32] Furthermore, it should be noted that the calculated M_n values from SEC are higher than the theoretical values, which is due to the used calibration (PS standards) as was previously demonstrated by comparison with MALDI-TOF MS.[32]

Table 1. Molar Masses and Polydispersity Indices Determined by SEC

	PMeOx		PEI	
M/I	M_n (g/mol)	PDI	M_n (g/mol)	PDI
5	3000	1.10	2500	1.06
10	4100	1.06	4900	1.05
20	5400	1.06	6800	1.09
30	6900	1.07	9900	1.08
40	8300	1.09	10900	1.12
50	9600	1.13	13900	1.12
60	10700	1.14	15200	1.15
80	12900	1.21	16800	1.21
100[a]	14900[a]	1.25[a]	21100[a]	1.16[a]
150	18900	1.41	27400	1.27
200	23700	1.40	28000	1.30

[a] Starting material poly(2-ethyl-2-oxazoline) instead of poly(2-methyl-2-oxazoline).

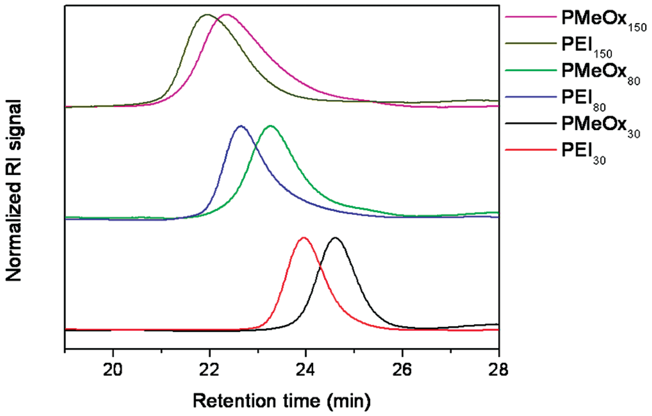

Figure 3. Selected SEC traces for the starting PMeOx and the final PEI with varying lengths.

The complete series of PMeOx polymers were subsequently utilized for the acidic hydrolysis. After hydrolysis, the resulting PEIs were analyzed by ^{1}H NMR spectroscopy to determine whether all PMeOx side groups were successfully cleaved. For all presented polymers the PMeOx side groups were removed in more than 99% yield, and ^{13}C NMR spectroscopy showed one characteristic peak indicative for linear PEI (δ = 49.5 ppm; results not shown).

SEC analysis revealed the molar masses and polydispersity indices of the obtained linear PEI, and the values are listed in Table 1. The PDI values for the starting PMeOx polymers and the final PEI polymers are comparable, except for PEI with 100, 150, and 200 units which show a lower PDI value in comparison to the starting PMeOx. Furthermore, it can be observed that the molar masses of PEI are slightly higher compared to the PMeOx starting polymers. For a better comparison, representative SEC curves from three different polymer lengths are depicted in Figure 3, showing the polymer signal before and after the acidic hydrolysis.

Although the molar mass of PEI is lower than for the starting PMeOx, the SEC signal indicates the opposite. This is most likely caused be the different solubility and/or hydrogen-bonding ability of the two polymers in hexafluoroisopropanol (SEC eluent), causing a different hydrodynamic volume of the two polymers. These results once more indicate that care should be taken upon interpretation of molar mass data from SEC based on calibration standards. Nonetheless, it is evident that the peak shape is not significantly changing after the acidic hydrolysis, indicating that the polymer backbone is staying intact. To further prove the successful synthesis of linear PEI, MALDI-TOF MS was performed for PEI with an aimed M/I ratio of 20. The complete MALDI-TOF MS spectrum can be observed in Figure 4 (left), and the corresponding Figure 4 (right) depicts a zoomed section of the mass spectrum with the peak assignment of the four different distributions.

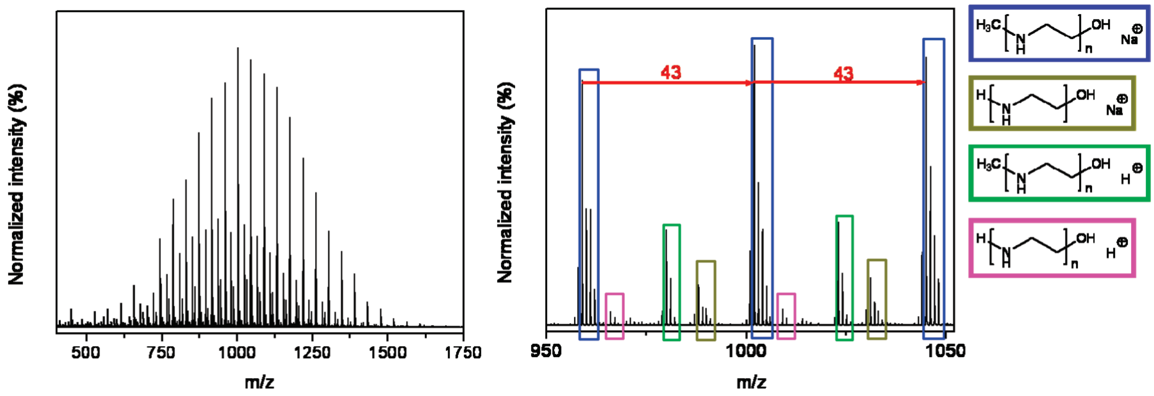

Figure 4. Left: mass spectrum (obtained by MALDI-TOF MS) for PEI with a M/I ratio of 20. Right: expanded region of the MALDI-TOF MS spectrum (*m*/*z* 950 to *m*/*z* 1050) with structural assignments of the different distributions.

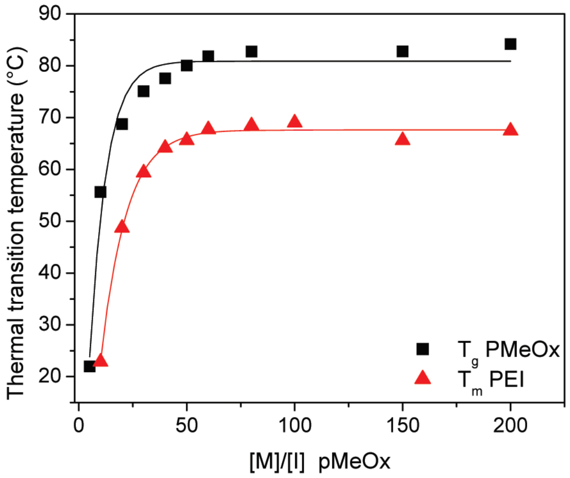

Figure 5. Glass-transition temperatures and melting temperatures for PMeOx and PEI polymers, respectively, with varying chain lengths, plotted versus the M/I ratio of the PMeOx starting polymers.

Two of these distributions belong to the desired product (methyl as starting group and hydroxyl as end group) with a proton or a sodium cation. The other two distributions are fitting to the side product from chain transfer during the polymerization (proton as starting group and hydroxyl as end group) also with a proton or a sodium cation. Because of the neutralization with NaOH after the acidic hydrolysis, the two distributions charged with sodium cation are visible. The calculated molar mass of the studied sample was measured to be 1020 g/mol with a PDI of 1.07, resulting in a DP of 24. The DP calculated from MALDI-TOF MS is in very good agreement with the M/I ratio of 20 used for the synthesis of the PMeOx. Furthermore, the peak assignments in Figure 4 (right) indicate an expected spacing of 43 Da. However, further reliable molar massas of the other PEI samples could not be determined by MALDI-TOF MS due to the limited ionization and/or desorption ability of the higher molar mass linear PEIs.

Properties of Linear PEIs. To elucidate selected structure−property relationships of PEI, the thermal and solubility properties were investigated. It is known from the literature that PEI is crystalline and therefore exhibits a melting temperature. Saegusa et al.[17] reported a $T_{m,onset}$ of 58.5 °C for PEI while the starting PMeOx is an amorphous polymer. The thermal properties for both PMeOx and PEI series were determined by differential scanning calorimetry (DSC). The second heating curves obtained with a heating rate of 20 K/min were analyzed for their T_g or T_m value, which are depicted in Figure 5. For all PMeOx polymers with various lengths a clear glass-transition temperature could be observed which is increasing up to a M/I ratio of 100. It is known that only for relatively short polymers the glass-transition temperature is increasing with the degree of polymerization, due to the diminishing effect of the more flexible chain ends and the end groups as explained in many polymer textbooks, which is also observed in Figure 5. The T_g for PMeOx with M/I ratio 100 was reported to be 80 °C,[37,13] which agrees well with the obtained value of 82 °C in Figure 5. For PEI a similar trend is observed for the melting temperature; again after a M/I ratio of 100 the melting temperature is stabilizing around 68 °C (peak temperature). Saegusa et al.[17] earlier reported a lower melting temperature of 58.5 °C, which is due to the determination method since Saegusa reported the onset of the melting peak while we determined the peak maximum. The large difference in thermal properties of the PMeOx and PEI can be related to the molecular structure. The PMeOx has relatively rigid amide groups, resulting in a relatively high T_g, while the methyl side chains prohibit close packing of the amide moieties, explaining the absence of crystallization. In contrast, the PEI is more flexible compared to PMeOx due to the absence of the amide moieties. However, strong hydrogen bonds can be formed between the NH groups, resulting in crystallization. It is rather surprising that the effects of chain ends on both the T_g of PMeOx and the T_m of PEI are very similar, resulting in similar evolution of T_g and T_m with increasing M/I, i.e., decreasing number of chain ends.

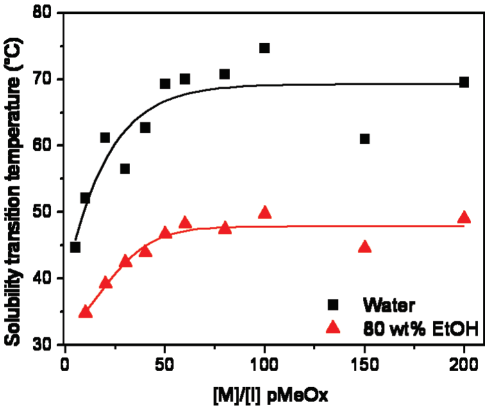

Figure 6. Solubility transition temperatures of PEI in water (5 mg mL^{-1}) and in 80 wt % EtOH solution (5 mg mL^{-1}) plotted versus the M/I ratio of the PMeOx starting polymers.

Furthermore, the solubility of the linear PEI series was investigated in water. Saegusa et al. noticed in their report that PEI is only soluble in water at elevated temperatures.[17] Therefore, we investigated the cloud point temperatures of all the synthesized linear PEIs with varying length. For all measurements a concentration of 5 mg/mL was utilized in water or water/ethanol mixtures. The cloud point temperatures upon heating were determined by measuring the turbidity of the polymer samples in water or water/ethanol mixtures as a function of temperature in the range from 1 to 100 °C. The results are depicted in Figure 6 for water and the water/ethanol solution containing 80 wt % ethanol, demonstrating that the dissolution temperature is increasing upon increasing the M/I ratio of the polymer.

Chantani et al.[27] explained the insolubility of linear PEI in water at temperatures below 60 °C by the formation of crystalline hydrates. In contradiction to branched PEI, which remains soluble in water even at low temperature, linear PEI has a strong tendency to crystallize, which is typical for perfectly linear, unsubstituted, flexible macromolecules. Moreover, the formation of hydrates through hydrogen bonding seems to favor the crystallization behavior of linear PEI as well.[38] Therefore, at elevated temperatures, the hydrated crystals melt, causing dissolution of the polymer in solution. For example, in water, the solubility transition to a soluble state of PEI with a M/I ratio of 200 occurs at 70 °C, while the melting temperature measured with DSC is 67 °C. It seems that for all samples the solubility transition temperature is in good agreement with the measured thermal transition temperature. This also explains the increase in dissolution temperature upon increasing the M/I ratio of the polymer. The polymers with shorter chain length have a lower melting temperature, indicating less stable crystals, and therefore, a lower temperature is required to dissolve the polymers. As such, the cloud points can be directly related to the T_m of the PEI. Moreover, as can be observed, the dissolution temperatures in water are much

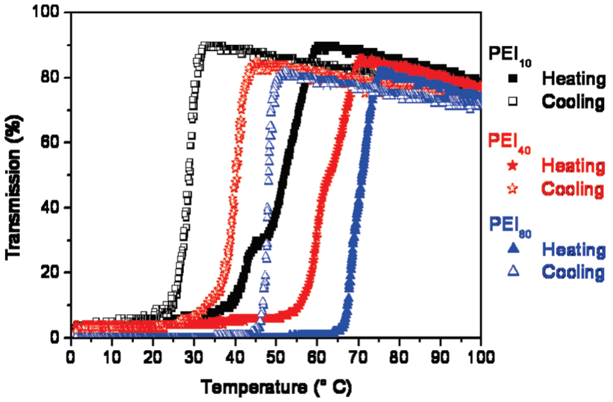

Figure 7. Selected transmittance vs temperature plots for PEI with respectively 10, 40, and 80 units at 5 mg mL^{-1} in water, demonstrating the hysteresis of the solubility transitions.

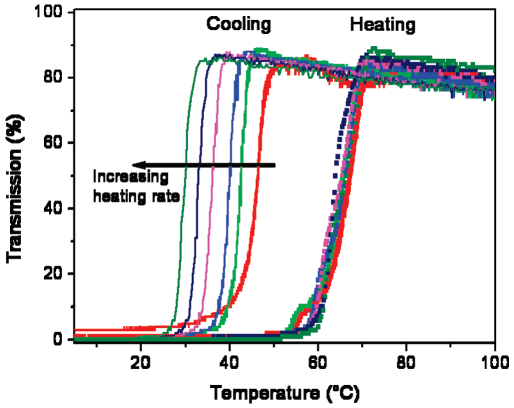

Figure 8. Selected transmittance vs temperature plots for PEI with a M/I ratio of 40 at 5 mg mL^{-1} in water with heating and cooling ramps of 0.1, 0.5, 1, 2, 3, and 5 °C min^{-1}.

more scattered compared to the cloud point temperatures in 80 wt % ethanol solution. This observation can be explained by the different crystallization behavior of PEI in the different solvent mixtures. In the solution containing ethanol, the overall solubility of PEI is increasing and the crystals are less stable, resulting in a lower dissolution temperature and less scattering between the data points.

Furthermore, the reversibility of the solubility transitions was investigated, and selected transmission versus temperature curves are shown in Figure 7. Sharp transition curves were found for all reported PEIs, but the solubility transition for heating and cooling shows a strong hysteresis and differs several tens of degrees in temperature. Upon cooling, the hydrated crystals form again, which occurs at a much lower temperature in solution. A closer look at the dissolution curves upon heating reveals two slopes for all three depicted polymers. Apparently, the dissolution of the crystals is a two-step process. The nature of this two-step process is not clear but might be due to the presence of smaller and larger crystals or to easier dissolution of the outer part of the crystals. Furthermore, the large hysteresis can additionally be explained by the formation of intermolecular hydrogen bonding, which plays a role in the formation of the hydrate crystals and which is also responsible for the hysteresis observed for poly(*N*-isopropylacrylamide).[39]

The hysteresis of PEI with a M/I ratio of 40 was investigated in more detail by varying the heating and cooling ramp. The ramps were varied from 0.1 to 5 °C min^{-1}, and as could be observed in Figure 8, the dissolution of the polymer in solution occurs for all heating ramps at about the same temperature, namely the melting temperature of the polymer (64.1 °C). Upon cooling, the crystals form again but at a much lower temperature as already observed in Figure 7, but when varying the cooling ramp the cloud point temperature is decreasing with faster cooling ramps. This proves that indeed PEI crystals form again at the cloud point temperature since it is known that the crystallization temperature is strongly dependent on the cooling ramp.

Conclusions

In conclusion, the cleaving kinetics of water-soluble poly(2-oxazoline)s, PEtOx, and PMeOx as well as structure−property relationships for linear PEI are reported. PEtOx is linearly cleaved in time. The length of the polymer was found to have no significant influence on the kinetics, indicating that all side chains are equally accessible during the acidic cleavage. Furthermore, PMeOx side groups could be cleaved faster due to less sterical hindrance as well as better hydration. The polymer concentration did not influence the hydrolysis rate, which can be ascribed to the large excess of hydrochloric acid and water.

In addition, a series of linear PEI were synthesized with varying M/I ratio with a cleavage efficiency higher than 99%. SEC measurements demonstrated comparable signals for PMeOx as well as the corresponding PEI, indicating that the polymer backbone is staying intact as was also confirmed by MALDI-TOF MS analysis. Thermal measurements revealed a T_g for all PMeOx polymers that increased up to a M/I ratio of 100. PEI, on the other hand, is crystalline and exhibits a melting temperature that shows a similar trend with M/I ratio as the T_g of PMeOx. Finally, solubility measurements in water and water/ethanol mixtures showed a crystallization-induced phase separation for PEI. The solubility transition to a soluble state was observed to correspond to the melting temperature, indicating that the PEI crystals need to melt in solution before solubilizing. Upon cooling, large hysteresis is observed due to extensive intermolecular hydrogen bonding, which may also play a role in the formation of the hydrate crystals. When increasing the amount of ethanol, the solubility of PEI is increasing, causing the solubility transition temperature to decrease accordingly.

The reported kinetics for the hydrolysis of PMeOx and PEtOx might form the basis for further investigations on (partial) selective hydrolysis of copolymers. In addition, the reported structure−property relationships for PEI opens up broader applications of these materials.

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