Poly(2-ethyl-2-oxazoline-co-N-propylethylene imine)s by controlled partial reduction of poly(2-ethyl-2-oxazoline)

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Abstract
The partial reduction of poly(2-ethyl-2-oxazoline) was investigated. A series of poly(2-ethyl-2-oxazoline-co-N-propylethylene imine)s were synthesized by direct reduction using lithium aluminum hydride or borane/dimethylsulfide (BH₃/DMS), respectively. It is shown that the degree of reduction can be readily controlled either by the reaction time when using an excess of LiAlH₄ or by the stoichiometry of BH₃/DMS as was demonstrated by ¹H-NMR spectroscopy. Differential scanning calorimetry revealed that the glass transition temperature of the products decreased with increasing degree of reduction up to 25% of reduction, above which no glass transition could be detected. This control over the reduction allows the tailor synthesis of partially cationic polymers, which, in combination over the hydrophilic/lipophilic balance through the side chain length allows a tight control over materials properties. Such materials may be interesting, inter alia, for biomaterials or organic electronics.
**Introduction**

Poly(ethylene imine) (PEI) is a widely used cationic polymer that can be prepared in two different architectures, linear and branched. PEI is used as a flocculant and as a retention aid in paper production or in textile industry. In the biomedical community, PEI is better known for its use as transfection agent\(^1\)\(^2\) or as protein/nucleic acid precipitation agent\(^3\).

While the branched PEI version is prepared by cationic polymerization of aziridine (Scheme 1a), the linear version is prepared in two steps. The first step is cationic ring-opening polymerization of 2-oxazolines. The second step is the hydrolysis of the intermediate non-ionic poly(2-oxazoline) which yields linear PEI (Scheme 1b). Poly(2-oxazoline) can also be transformed into cationic polymer by another strategy, which has been much less investigated. The amide motif in the repeating unit can be reduced to a tertiary amine (Scheme 1c).\(^4\)\(^5\) The resulting polymers are known as poly(N-alkylethylene imine)s (PAEI). PAEI can also be obtained by alkylation of PEI employing the Leuckart-Wallach reaction. To date, this has been realized only for methylation using the Eschweiler-Clarke reductive N-methylation. In fact this approach predates the amide reduction.\(^6\)\(^7\)

Besides the difference of secondary (PEI) and tertiary amine (PAEI), the PAEI are chemically much more versatile by virtue of the N-substituent. Interestingly, while thousands of scientific reports deal with PEI one way or the other, hardly more than a handful of reports dealing with synthesis\(^4\)\(^7\), properties\(^5\)\(^8\) and use\(^8\)\(^9\) of PAEI can be found in the literature and these few reports have received remarkably little attention.

More recently, Fukuda et al. investigated the solution properties of poly(N-methylethylene imine) synthesized via the Eschweiler-Clarke methylation.\(^8\) They reported that this material is interesting as water-soluble polycationic material.

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**Scheme 1**: a) Reaction scheme for the preparation of a) branched and b) linear poly(ethylene imine). c) Reaction scheme for the (partial) reduction of poly(2-ethyl-2-oxazoline)s to poly(N-propyl ethyleneimine).
To the best of our knowledge, all previous reports in the context of PAEI discussed the synthesis and properties of PAEI homopolymers. However, partial reduction of POx should lead to copolymers of POx and PAEI (P(Ox-co-AEI)), but this has not been described to date. This contribution investigates the partial reduction of POx using two different reducing agents, BH$_3$ and LiAlH$_4$. The partially reduced POx were characterized via $^1$H-NMR spectroscopy, differential scanning calorimetry and acid-base titration.

**Materials and Methods**

**Materials**
All chemicals and reagents were purchased from Sigma-Aldrich or Acros and used as received unless stated otherwise. The poly(2-ethyl-2-oxazoline) had a molar mass of 50 kg/mol and a dispersity of 3-4 (SiAl product number #372846).

**Methods**
Reduction with LiAlH$_4$
A previously reported procedure described by Kobayashi was slightly altered. We employed a THF solution of LiAlH$_4$ instead of diethyl ether, as PEtOx is insoluble in Et$_2$O. In a 50 ml Schlenk flask equipped with a magnetic stir bar 500 mg poly(2-ethyl-2-oxazoline)$_{500}$ (PEtOx) (10 µmol) were dissolved in 20 ml dichloromethane. A 1.2 M LiAlH$_4$ solution/dispersion in THF was added slowly while cooling with an ice bath. The reaction mixture was refluxed for 3 d, if a complete reaction was the aim. Shorter time periods were used when the degree of reduction was to be adjusted by reaction time.

Reduction with BH$_3$/DMS
A procedure introduced by Perner and Schulz and adapted by Hoogenboom et al. was slightly modified. In a 100 ml Schlenk flask equipped with a magnetic stir bar 500 g PEtOx were dissolved in 50 ml THF. Depending on the aimed degree of reduction, a corresponding amount of a 2.715 mmol·g$^{-1}$ BH$_3$/DMS solution in THF was added while cooling with an ice bath. The reaction mixture was refluxed for 3 d and subsequently, the solvent was removed *in vacuo*. To the residue 50 ml of methanol was added and refluxed overnight. The solvent was removed using a rotational evaporator.

**NMR analysis of the reduction degree**
NMR spectra were recorded on a Fourier 300 (1H; 300.12 MHz), Bruker Biospin (Rheinstetten, Germany) at 298 K. The spectra were calibrated to the signal of residual protonated solvent (CDCl$_3$: 7.26 ppm). For the determination of the degree of reduction, signals at 1.12 and 0.85 ppm were employed.

**Differential scanning calorimetry**
For DSC studies samples were placed into flat-bottom aluminum pans with crimped-on lids and heated/cooled on a calibrated DSC 204 F1 Phoenix equipped with a CC200 F1 Controller, (NETZSCH, Selb, Germany). The dynamic scans were recorded in nitrogen atmosphere with a heating rate of 5 °C/min (0 ° – 180 °C).
Results and Discussion

Different reagents can be used for the reduction of tertiary amides to amines. For the reduction of POx to PAEI, Saegusa\(^4\) and Hoogenboom\(^5\) described the use of LiAlH\(_4\) and BH\(_3\)/DMS, respectively. In these reports, the POx was always reduced fully to the PNAEI. The purpose of the present study was to investigate the partial reduction of poly(2-ethyl-2-oxazoline) (PEtOx) to poly(2-ethyl-2-oxazoline-co-N-propylethylene imine)s (P(EtOx-co-NPrEI)). The degree of reduction was determined using \(^1\)H-NMR spectroscopy. The \(^1\)H-NMR spectra of PEtOx is characterized by three main signal groups, the backbone protons give rise to a signal around \(\delta = 3.4-3.5\) ppm, the side chain methylene group at \(\delta = 2.2-2.5\) ppm and the methyl group at \(\delta = 1.0-1.2\) ppm (Figure 1, Signals A, B and C). The PNPrEI gives rise to signals at \(\delta = 2.6\) ppm of the backbone protons, at \(\delta = 2.4\) ppm the side chain methylene group adjacent to the nitrogen while at \(\delta = 1.4\) ppm appear the signals attributed to the central methylene group of the propyl side chain (Figure 1, Signals D, E and F). Finally, the side chain methyl group gives rise to signals at \(\delta = 0.8\) ppm (Figure 1, Signal G). As can be expected, the partially reduced samples show more complex \(^1\)H-NMR spectra (Figure 1). For the determination of the degree of reduction, the two signals of the methyl groups in the side chains at \(\delta = 1.1\) ppm and \(\delta = 0.8\) ppm, respectively seem to be particularly suited. The degree of reduction was calculated as the ratio of the \(I_G/(I_G+I_C)\).

We decided to investigate the reduction of PEtOx with both previously reported reducing agents. Initially, we studied whether the reduction could be controlled by the stoichiometry of the added LiAlH\(_4\). Important to note, one eq. of LiAlH\(_4\) is able to reduce 2 eq. of amide.\(^12\) This strategy does allow partial reduction of POx, but only with limited degree of control. In four different reactions, we added 30\%, 30\%, 50\%, 70\% and 150\% of reduction equivalents (with respect to amide groups in the polymer) of LiAlH\(_4\). After 68h at 313 K, the degrees of reduction, determined by \(^1\)H-NMR were 3, 6, 25, 46\% and 100\%, respectively (Table 1). In the first experiment carried out in THF, we observed phase separation of a gel-like material during the reaction. This can be attributed to the reduced solubility of the partially reduced POx. Therefore, and in accordance to earlier reports, the following experiments were carried out in dichloromethane. However, also in the first experiment with DCM, we observed precipitation, presumably because the concentration was too high. Subsequent experiments were carried out at a lower concentration and no precipitation was observed. The precipitation presumably is also the reason for the very low degree of reduction observed in the first experiments, which was much lower than expected from the stoichiometry. However, also in the absence of precipitation, the determined degree of reduction did not correspond well with the stoichiometry.
Table 1 | Partial reduction of PEtOx with sub-stoichiometric amounts of LiAlH₄ at 313 K using sub-stoichiometric amounts of BH₃/DMS refluxed at 343 K.

<table>
<thead>
<tr>
<th>NMR trace (Figure 1)</th>
<th>[LiAlH₄]/[amide] (red. eq./amide)</th>
<th>Solvent</th>
<th>I₀/(I₀+Iᵣ)</th>
<th>NMR trace (Figure 3)</th>
<th>[BH₃]/[amide]</th>
<th>I₀/(I₀+Iᵣ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.15 (0.3)</td>
<td>THF</td>
<td>0.03</td>
<td>2</td>
<td>0.10</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>0.15 (0.3)</td>
<td>DCM</td>
<td>0.06</td>
<td>3</td>
<td>0.30</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>0.25 (0.5)</td>
<td>DCM</td>
<td>0.25</td>
<td>4</td>
<td>0.40</td>
<td>0.41</td>
</tr>
<tr>
<td>7</td>
<td>0.35 (0.7)</td>
<td>DCM</td>
<td>0.46</td>
<td>5</td>
<td>0.50</td>
<td>0.47</td>
</tr>
<tr>
<td>16</td>
<td>0.75 (1.5)</td>
<td>DCM</td>
<td>&gt;0.99</td>
<td>6</td>
<td>0.75</td>
<td>0.79</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Nevertheless, these first experiments showed clearly that partial reduction of POx can be used to obtain the desired P(EtOx-co-NPrEI) copolymers. It is known from literature that sub-stoichiometric use of LiAlH₄ can lead to C-N bond scission and formation of alcohols or aldehydes, which, in our case is synonymous with side chain scission. Indeed, in the NMR of the latter two reactions, we do see some evidence of the side product 1-propanol in the ¹H-NMR spectra (Fig. 1, red arrowheads). However, in the majority of cases, we found no evidence of this side reaction.

Figure 1 | a) Waterfall plot of NMR spectra in CDCl₃ of reactions with LiAlH₄ depicted in Tables 1 and 2. b) Detailed view of the two signals at 1.12 ppm and 0.85 ppm, which were used for the determination of the degree of reduction.

In a second set of experiments, we added an excess of LiAlH₄ (1.5 eq per amide) and quenched the reaction after predetermined times (313K, 0.5, 1, 2, 4, 24 and 68h) by addition of an excess of water. After workup, NMR was again used to determine the degree of reduction. We realized that even after only 30 min the reduction was almost quantitative and after 1 h only signals of the fully reduced PNPrEI were observed (data not shown). Interestingly, Micovic and Mihailovic already
reported in 1955 that the reduction of tertiary amides is very fast in spite of a majority of researchers conducting the reaction for prolonged times.\textsuperscript{12}

Therefore, we investigated the kinetics of the reduction by conducting the PEtOx reduction directly in the NMR tube at 295 K. For this, 5 mg of LiAlH\textsubscript{4} were dispersed in 0.1 mL of THF-d\textsubscript{8} in a screwcap NMR tube and 10 mg of PEtOx dissolved in 0.6 mL dichloromethane were added. Immediately, NMR spectra were acquired over the course of 300 min. Under these conditions, again, a very fast reduction of the polymer was observed. Even at the earliest measurement (\approx 3 min), the degree of reduction was already more than 20\% and after 60 min, the degree of reduction reached 90\% (Figure 2). For 50\% reduction, the reaction time under these conditions was approx. 10 min. Notably, no formation of 1-propanol was observed.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{^{1}H-NMR kinetic investigation of the reduction of PEtOx using an excess of LiAlH\textsubscript{4} at 298 K. The degree of reduction was determined using the signals of the terminal methyl groups in the side chains at 1.1 ppm (PEtOx) and 0.8 ppm (PNPrEI), respectively.}
\end{figure}

Hoogenboom and co-workers reported on the exhaustive reduction of POx, including PEtOx using borane/dimethylsulfide (BH\textsubscript{3}/DMS)\textsuperscript{5} following a procedure reported by Perner and Schulz.\textsuperscript{11} Accordingly, we investigated the partial reduction using this reagent. Similar as with LiAlH\textsubscript{4}, we investigated the use of substoichiometric amounts of reducing agent to control the partial reduction of POx (Table 2). Again, the products were characterized by ^{1}H-NMR (Figure 3). In contrast to the situation with LiAlH\textsubscript{4}, BH\textsubscript{3}/DMS seems to be well suited to control the partial reduction via the stoichiometry. The calculated degrees of reduction corroborated reasonably well with the targeted values. Thus, the degree of partial reduction could be controlled from 10\% to about 80\%. 

\begin{table}[h]
\centering
\caption{Partial reduction of POx using BH\textsubscript{3}/DMS.}
\begin{tabular}{|c|c|}
\hline
Reduction & Degree of reduction (\%) \\
\hline
10 & 10 \\
20 & 20 \\
30 & 30 \\
40 & 40 \\
50 & 50 \\
60 & 60 \\
70 & 70 \\
80 & 80 \\
\hline
\end{tabular}
\end{table}
Hoogenboom and co-workers compared the thermal properties of POx and PNAEI by means of differential scanning calorimetry. In the case of fully reduced PEtOx, i.e. PNPrEI, they reported that neither $T_g$ nor $T_m$ could be detected. Since PEtOx is well known to have a $T_g$ of around 330 K, we were curious how the $T_g$ changes with the degree of reduction. We found that at 10% reduced side chains, the $T_g$ shifted to 313 K. Increasing the degree of reduction further to 25%, the $T_g$ decreased to 293 K. Samples with an even higher degree of reduction ($\geq 40\%$) did not show a $T_g$ in our experimental setup, which agrees well with the observation by Hoogenboom and co-workers.

Figure 3] $^1$H-NMR spectra of PEtOx (trace 1), partially (P(EtOx-co-NPrEI, traces 2-6) and fully reduced PEtOx (PNPrEI, trace 7) with BH$_3$/DMS at 343 K.

Figure 4] DSC heating curves of poly(2-ethyl-2-oxazoline) (PEtOx) and partially reduced samples with a degree of reduction of 0.1, i.e. P(EtOx$_{0.9}$-co-NPrEI$_{0.1}$) and 0.25, i.e. P(EtOx$_{0.75}$-co-NPrEI$_{0.25}$).
As many water-soluble polymers do, the water solubility of several POx and PEtOx in particular shows a dependence on the temperature. Depending on the molar mass and the polymer architecture, the lower critical solution temperature of PEtOx ranges from 343 to about 370 K. Hoogenboom et al. reported that PNPrEI is insoluble in water, which shows the influence and importance of the tertiary amide motif in POx for the solubility. It will be interesting to study the influence of partial reduction on the lower critical solution temperature, but for the present contribution, we were more interested in studying the buffering capacity of the partially reduced POx. This is a relevant property for a number of application, for example for the complexation of nucleic acids during the formation of polyplexes for gene delivery or complexation of proteins in nanozymes. Accordingly, we investigated the acid-base titration of solution of PEtOx (as control) as well as partially P(EtOx\_1-x-co-NPrEl\_x) and fully reduced PNPrEI. The polymers were dissolved in 0.1 M HCl and titrated against 0.1 M NaOH (Figure 5).

Important to note, PNPrEI was fully soluble at 2 mg/mL in 0.1 M HCl but precipitation was observed starting at a pH of 8, while in all other cases, the solutions remained clear throughout the titration experiment. As can be expected, PEtOx does not show any effect in the acid base titration (Figure S2) as tertiary amides are not ionized between pH 2 and 12. It should be noted that Hsiue and co-workers mention a pH responsive character of POx, but this seems unlikely to be due to any protonation of tertiary amides. The effect observed may have been due to end-groups or other impurities. In contrast to POx, all reduced samples clearly show a buffering capacity during the potentiometric pH titration. As might be expected from tertiary amines, the exhaustively reduced PNPrEI shows a pK\_a value between 8 and 9. As mentioned before, in this pH range the polymer also precipitated. Interestingly, the pK\_a values appears to be strongly influenced by the degree of reduction, clearly hinting at cooperative effects, or neighbor group effects within the polymer as has been described in different polyelectrolytes including poly(vinylamine). At 80% reduction, the pK\_a remains largely unchanged. However, the titration curve appears to broaden. This broadening becomes much more pronounced for the polymers with reduction degrees between 30% and 50%. In contrast, at 10% reduction degree, the titration curve is quite similar to the one at 100% reduction, only the pK\_a value appears to be about 1-2 units lower. The polymer with every second repeat unit (statistically) reduced shows a very interesting titration behavior showing a practically linear behavior between pH 3.5 and 9.5. The titration curve reflects to some extent the titration curve of branched PEI as reported by Andresen and co-workers. This is particularly interesting as this effect is not observed as the degree of reduction is increased further. The preliminary results presented here warrant a much more detailed study, that includes different side chains and a more detailed investigation of the apparent pK\_a values at different reduction degrees. However, it is immediately apparent that such broad buffering capacity of P(EtOx\_0.5-co-NPrEl\_0.5) could be very interesting for a range of applications, including non-viral vectors for gene therapy. Particularly interesting in this context is that the broad buffering capacity is observed at intermediate charge densities. This may have major implications on the cytotoxicity of the corresponding materials which we will need to evaluated. Hsiue et al. as well Hoogenboom et al. demonstrated
that partially hydrolyzed POx exhibit much lower cytotoxicity compared to PEI which may be similar for partially reduced POx.

**Figure 5** Titration curves at different degrees of reduction, ranging from 0 – 100 %. Polymers were dissolved in 0.1 M HCl and titrated with 0.1 M NaOH. The titrations were carried out in duplicate or triplicate. The reference titration is in dotted lines, the individual titration curves are shown in black solid lines while the average is red solid lines. In the case of the copolymers with a degree of reduction of 27%, the polymer concentration was not consistent between the three titrations, therefore calculation of the average was not performed.

**Conclusion**

In conclusion, we could show that the controlled reduction of poly(2-ethyl-2-oxazoline)s can be used to prepare poly(2-ethyl-2-oxazoline-co-N-propylethylene imine)s as well as poly(N-propylethylene imine)s after exhaustive reduction. This is the first report showing the synthesis and some properties of such copolymers. Considering the side chain variability of POx, we believe that our approach can be used to access a large variety of different copolymers varying in hydrophilicity and hydrophobicity and cationic charge density. Such materials could be very interesting in a large variety of applications, including applications as biomaterials or in organic electronics. Future studies will have to elucidate the effect of side chains on the rate of the reduction, similar to studies investigating the effects of POx side chains on the hydrolysis. Moreover, the influence of the degree of reduction on the lower critical solution temperature of the polymers will be interesting to study.

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References


