Defined High Molar Mass Poly(2-Oxazoline)s

Polymerization

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Defined High Molar Mass Poly(2-Oxazoline)s

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Abstract: *Poly(2-alkyl-2-oxazoline)s (PAOx) are regaining interest for biomedical applications. However, their full potential is hampered by the inability to synthesise uniform high-molar mass PAOx. In this work, we proposed alternative intrinsic chain transfer mechanisms based on 2-oxazoline and oxazolinium chain-end tautomerisation and derived improved polymerization conditions to suppress chain transfer, allowing the synthesis of highly defined poly(2-ethyl-2-oxazoline)s up to ca. 50 kDa (dispersity (Đ) < 1.05) and defined polymers up to at least 300 kDa (Đ < 1.2). The determination of the chain transfer constants for the polymerisations hinted towards the tautomerisation of the oxazolinium chain end as most plausible cause for chain transfer. Finally, the method was applied for the preparation of up to 60 kDa molar mass copolymers of 2-ethyl-2-oxazoline and 2-methoxycarbonylethyl-2-oxazoline.*

The synthesis of poly(2-alkyl-2-oxazoline) (PAOx), at that time referred to as poly(*N*-acylaziridine)s or poly(*N*-acylethylenimine)s, was discovered just over 50 years ago.[1–5] The cationic ring-opening polymerization (CROP) of 2-oxazolines was found to proceed with electrophilic initiators. Importantly, the cyclic imino ether functionality isomerizes to a more stable amide structure during the polymerization (Scheme 1 a, propagation). This isomerisation is the driving force for the polymerisation,[6] and it leads to a large difference in nucleophilicity between the monomer and the resulting polymer allowing a wide range of polymerization temperatures without the occurrence of backbiting or chain transfer to polymer side reactions.[7]

The living CROP of 2-oxazolines allows the preparation of a wide range of defined polymers having different physicochemical properties, ranging, e.g., from very hydrophilic to hydrophobic and fluorophilic as well as from amorphous (both high and low glass transition temperatures) up to semi-crystalline with melting transition temperatures up to 300 °C.[8] Furthermore, (orthogonally) functionalized polymers can be prepared using functional initiators, terminating agents and/or functional monomers.[9]

The high tunability of the PAOx polymer structure in combination with its high biocompatibility and non-fouling behaviour have raised significant interest in the use of PAOx for biomedical applications. For a comprehensive overview of recent developments, the reader is referred to an excellent review by Luxenhofer et al.[10] The use of PAOx for biomedical applications has been established beyond proof-of-concept research as the first in human studies (Phase 1 a clinical trials) were successfully finished by Serina Therapeutics for a PAOx-drug conjugate.[11] Besides biomedical applications, PAOx are also popular for the development of responsive and smart materials as the monomer side chain and the copolymerization of different 2-oxazoline monomers allows accurate tuning of the overall hydrophilic-hydrophobic balance and, thus, the thermoresponsive lower critical solution temperature behaviour in water. This, in combination with the ease of end-group functionalization has led to multi-responsive PAOx,[12–14] as well as intricate thermoresponsive colour changing solutions.[15] Another recently emerging application of PAOx is their use as dipole layer in solar cells and LEDs.[16,17]

Whilst the polymerisation of 2-oxazolines has been established for many decades and numerous applications of PAOx have been developed, it has been impossible to prepare uniform high-molar mass PAOx.[18] Low molar mass polymers with very narrow dispersities have been synthesised,[19,20] but attempts to produce narrow polymers with molar masses beyond 10 kDa are rarely reported and involve relatively low temperatures, with Monnery et al. being the most significant reporting a poly(2-isopropyl-2-oxazoline with a number average molar mass (M_n) of 55.9 kDa and a dispersity ($Đ$) of 1.06.[21,22] Nonetheless, chain coupling became apparent and the initiator efficiency was below unity, indicative of side reactions.

The CROP of 2-oxazolines is highly sensitive for extrinsic nucleophilic impurities, such as water (even humidity from the air), ammonia (potential contaminant in the monomer), and potentially interfering solvents.[21,23–25] Even an apparently pure polymerization system, free of nucleophilic impurities, still undergoes some side-reactions. This indicates the occurrence of intrinsic side reactions. Litt suggested in 1975 that an E2 β-elimination reaction competes with the S_N2 propagation reaction, whereby the monomer acts as a base instead of a nucleophile (Scheme 1 b).[18] This hydrogen

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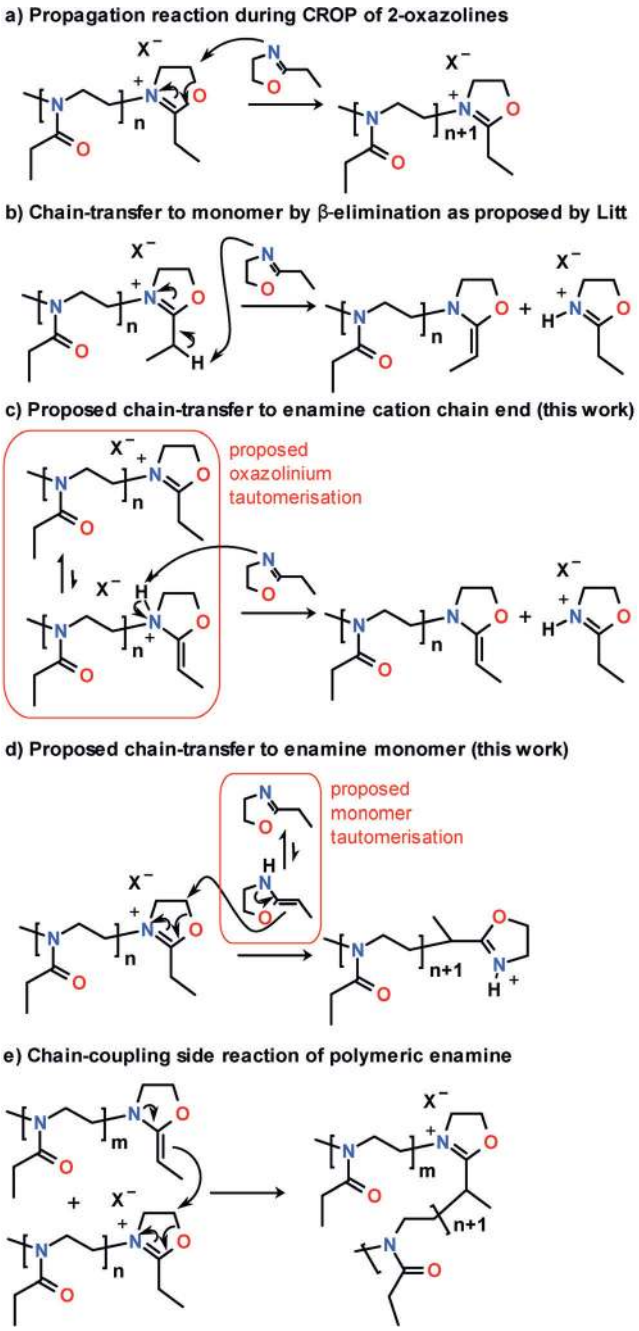

Scheme 1. Reactions in the polymerisation of 2-ethyl-2-oxazoline as model for 2-alkyl-2-oxazolines: a) Propagation via nucleophilic attack of the 2-oxazoline on the 2-oxazolinium chain end. b) Chain transfer to monomer via β-elimination (hydrogen abstraction). c) Proposed chain transfer via tautomerisation of the oxazolinium chain end. d) Proposed chain transfer via tautomerisation of the 2-oxazoline monomer. The final structure can undergo tautomerisation to the oxazolinium or chain transfer to monomer via the pathway shown in part (b). e) Coupling of the enamine terminated polymer to a living polymer chain.

abstraction leads to a slightly nucleophilic enamine end-functionalized "dead" polymer, and a new proton initiated chain. Being slightly nucleophilic, the "dead" enamine functionalized chains can react with "living" oxazolinium chain ends, especially at higher monomer conversion. During this chain coupling, the "dead" enamine chain end is converted back to a "living" oxazolinium species and mid-chain propagation may occur (Scheme 1e). Since the "dead" chains can take the livingness of another chain to live again they can be described as vampiric chains. Litt determined the chain transfer coefficients (CTCs) in high temperature polymerisations of unhindered higher 2-alkyl-2-oxazolines to be in the range of 1/100 to 1/300.[18]

Hence the synthesis of higher molar mass defined linear PAOx has been believed to be impossible. As a result, most research groups restricting their research to relatively lower molar masses with a degree of polymerisation (DP) below 200.

In this work, we first critically assess the intrinsic chain transfer reactions during the CROP of 2-oxazolines followed by the development of improved polymerization conditions for the preparation of defined high molar mass PAOx leading to poly(2-ethyl-2-oxazoline) (PEtOx) with a molar mass of 300 kDa and a $Đ$ below 1.2, previously believed to be unattainable.

When assessing the originally proposed β-elimination chain transfer mechanism, the acidity of the abstracted hydrogen may be questioned. Instead we hypothesize that tautomerisation of the monomer and/or cationic oxazolinium may play a role in the mechanism. Scheme 1c shows the proposed oxazolinium tautomerisation to the protonated enamine form. The presence of excess monomer may lead to proton transfer to monomer, thereby yielding the same chain transfer products as proposed in the original mechanism. The second proposed tautomerisation of the 2-oxazoline monomer is shown in Scheme 1d. It may be speculated that if the 2-oxazoline tautomerises to the enamine form, the nucleophilic attack of the enamine onto the living oxazolinium cation leads to a protonated oxazolinium end group. In a next step, this oxazolinium end group may either 1) act as living chain end upon monomer addition, leaving a secondary amide in the polymer chain or 2) it may undergo proton transfer to monomer to initiate a new chain while leaving an oxazoline end-functionalized macromonomer. This tautomerisation of the 2-ethyl-2-oxazoline (EtOx) monomer could be indirectly experimentally confirmed by deuteration of the side chain upon heating EtOx to 140 °C in [D_6]acetone (see the Supporting Information, Section S1) while at 40 °C no deuterium exchange was observed. Importantly, both the original mechanism from Litt and the monomer tautomerisation mechanisms should correlate to the monomer concentration while the oxazolinium tautomerisation should correlate to the concentration of living cationic chain ends as rate-limiting step.

The purity of the reaction mixture is a key factor to rule out extrinsic side reactions and to obtain a living CROP. Therefore, the liquid reagents and solvent were purified with ninhydrin, sodium and living polymer as appropriate. However, applying these highly purified reagents to the standard microwave polymerization conditions for the polymerisation of EtOx at 140 °C in acetonitrile using methyl tosylate as initiator did not result in uniform PEtOx (Section S2). Instead, low molar mass tailing and higher molar mass peaks are clearly present revealing the occurrence of chain

transfer and chain coupling side-reactions leading to a $Đ$ of 1.10 for PEtOx with a peak molar mass (M_p) of 16 kDa and a $Đ$ of 1.19 for PEtOx with M_p of 35 kDa. These results clearly indicate that further optimization of the polymerisation conditions is required to suppress the intrinsic side reactions.

The polymerization was performed at lower temperature to shift the potential tautomerisation equilibria towards the oxazoline and oxazolinium species. Furthermore, chlorobenzene was used as solvent, as it is completely non-interfering and it is known to be rate accelerating for the CROP of 2-oxazolines up to 80 °C due to ion pairing.[21] The used initiator was a bench stable oxazolinium salt, namely 2-phenyl-2-oxazolinium tetrafluoroborate, which ensures fast initiation and facilitates its use under high vacuum conditions (see Section S3 for the polymerization scheme). To ensure absolute absence of interfering impurities in the polymerisation mixture, the already highly pure reagents were purified again inside a high-vacuum system (black vacuum, $< 10^{-5}$ torr), using pre-exposure to the initiator salt before polymerisation. The further purified monomer and solvent are then transferred to the polymerization flask, containing the correct quantity of initiator for the polymerization, by static distillation under high vacuum. After distillation the polymerisation mixture is heated for the required time after which termination is induced by the addition of a large excess of nucleophile, in this case methanolic ammonia to produce an ω-primary ammonium (visible in ^{1}H NMR spectra, signal for the adjacent methylene at $\delta = 2.9$; see Section S4). The lyophilised polymers reacted with ninhydrin to slowly form Ruhemann's purple, and were successfully modified with isothiocyanates in the presence of a base confirming the presence of the primary amino end group, which is consistent with previous reports.[12,26–28]

When the polymerisations were performed at 40 °C it yielded PEtOx polymers with extremely narrow dispersity and minimal shoulders in the size exclusion chromatograms with a $Đ < 1.06$ up to a chain length of 58 kDa at high monomer conversion of $> 90\,\%$ (SEC; Figure 1, top; Table 1; see Table S1). Defined polymers with $Đ < 1.2$ were also obtained when targeting higher molar masses at ca. 50 % to avoid too high viscosity and too long reaction times (Figure 1, bottom; Table 1; see Table S2). The exact conversion in most cases could not be determined due to the high viscosity of the mixture. In line with expectations, the polymers of very high molar mass (DP > 2000) are still significantly more defined than polymers of DP = 100 produced by microwave polymerization.

The rather long polymerisation times for the preparation of these defined PEtOx, namely 20 days for 50 kDa at full conversion and 28 days for 300 kDa at 50 % conversion, are less than ideal. Since the oxazolinium remains predominantly ion-paired until ca. 80 °C it was decided to investigate whether low $Đ$ could be retained at slightly higher temperatures. Unfortunately, already at 60 °C the low molar mass tailing and higher molar mass peaks were much more apparent in SEC indicating a greater tendency to chain-couple, although the dispersity is only slightly increased at lower DP (see Section S7; Figure S5 and Table S3).

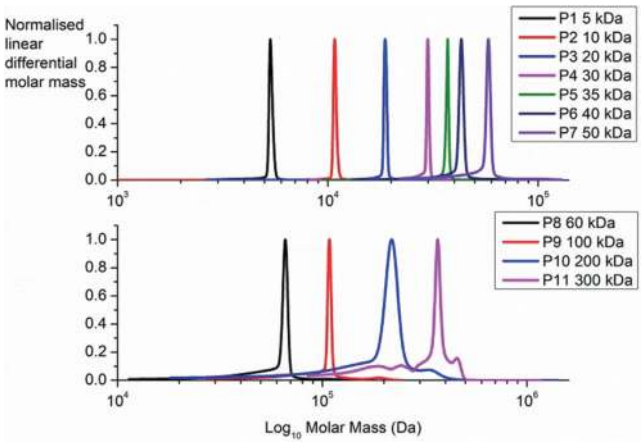

Figure 1. Size exclusion chromatograms of the as obtained PEtOx after termination with ammonia determined by size exclusion chromatography with a light scattering detector in DMAc with 50 mM LiCl. Top: Polymers with a molar mass up to 60 kDa, which narrow dispersity. Bottom: Higher mass polymers revealing broadened molar mass distributions due to unavoidable intrinsic chain transfer reactions. P11 has reached the apparent exclusion limit of the column, accounting for the peak shape.

Table 1: Molar masses and dispersity of the PEtOx polymers shown in Figure 1, obtained by SEC-MALS in *N*,*N*-dimethylacetamide with 50 mM LiCl.

Serial No.	M_n (kDa)	M_m (kDa)	M_p (kDa)	$Đ$
P1	5.3	5.3	5.3	1.01
P2	10.5	11.0	10.7	1.05
P3	18.0	18.8	18.7	1.04
P4	29.1	30.3	30.0	1.04
P5	35.7	36.7	37.0	1.03
P6	41.4	43.4	43.0	1.05
P7	54.3	57.6	57.9	1.06
P8	56.2	62.5	66.0	1.11
P9	95.2	107.6	108.7	1.13
P10	183.2	216.5	215.9	1.18
P11	287.4	330.5	366.1	> 1.15

From the above it is evident that the improved synthetic methodology allows the preparation of much better defined high molar mass PEtOx. Nonetheless, some intrinsic side reactions still occur. To verify whether this is due to the chain transfer followed by chain coupling, the obtained PEtOx was converted into poly(ethylenimine) by acidic hydrolysis. After hydrolysis the SEC revealed that the high molar mass peaks had disappeared (Section S8; Figure S6), as expected based on the amide linkage between the polymer chains after chain coupling (Scheme 1e) and propagation of the mid chain oxazolinium.

To evaluate which of the three proposed mechanisms is responsible for the intrinsic chain transfer reactions, the CTC was determined for the different polymerization via the chain length distribution (CLD). Therefore, the area of an undisturbed CLD was determined by fitting a curve to the major peak of the observed CLD (Section S9; Figure S7). The ratio of the integrals of the observed and fitted peaks gives the proportion of chain ends that are outside the major distribu-

tion due to a transfer event. Since each transfer event produces two chains outside of the major distribution the equation for the CTC becomes equation (1).

$$\mathrm{CTC} = 1 - \left(\frac{\Sigma\ (\text{fitted peak})}{\Sigma\ (\text{observed distribution})}\right)^{\left(\frac{1}{n \cdot \mathrm{DP}}\right)} \quad (1)$$

Herein, n is the number of chains outside the distribution created by chain transfer (i.e. 2 in this system) and DP is assumed to be M_p divided by the molar mass of the repeat unit. The results are summarized in Table S4. The obtained CTC values are at least an order of magnitude lower than previously reported values, with the lowest values being ≈ 1/6700 for the highest DP polymers (P10 and P11).

When the calculated CTC is plotted versus the concentration of oxazolinium chain ends in the system (theoretical concentration of initiator assuming no termination), there is a strong relationship (Figure 2; Figure S8). The lower the concentration of oxazolinium ions (i.e. the higher the target DP), the lower the CTC. This general trend also holds true for different monomer concentrations indicating that chain transfer is not governed by the monomer concentration, but rather by the concentration of living chain ends. As only the proposed chain transfer mechanism via tautomerisation of the oxazolinium chain end follows this dependence (Scheme 1c), it may be concluded that chain transfer is induced by oxazolinium tautomerisation rather than β-elimination by direct hydrogen abstraction. A direct correlation of 1/CTC with concentration of living ends was also found for the CROP at 60 °C, albeit with a steeper slope indicating (slightly) more pronounced chain transfer in agreement with the shape of the SEC traces.

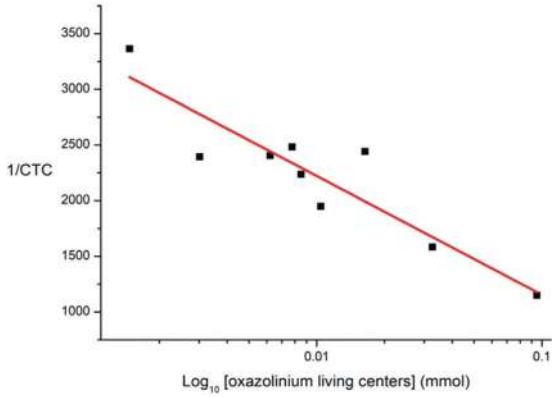

Figure 2. Relationship of log (concentration of living ends) and 1/CTC for polymerisations at 40–42 °C. P-value = 0.0025.

To demonstrate that the developed synthetic methodology is not only applicable to EtOx, but also for other monomers, a series of functional copolymers was prepared based on EtOx with 2-methoxycarbonylethyl-2-oxazoline (MestOx) as methyl ester containing comonomer. After a slight modification of the procedure, that is, heating of the set-up during static distillation to transfer the higher boiling MestOx, highly defined copolymers containing 3–10 mol % of MestOx were obtained with an M_p up to 66 kDa and a $Đ$ of 1.11 (Section S11).

In conclusion, we have demonstrated a polymerisation method for the CROP of 2-oxazolines with an at least 10 times lower chain transfer coefficient than commonly utilised systems. This allows the synthesis of previously unattainable defined high molar mass PAOx. The determination of the CTCs allowed use to analyse the mechanisms of chain-transfer revealing that it is related to tautomerisation of the oxazolinium cationic chain end rather than monomer concentration, at least at the low polymerization temperature used in this study. At high polymerization temperature, it is plausible that tautomerisation of the monomer also plays a role as we have been able to indirectly prove that this occurs at 140 °C.

The accessibility of uniform high molar mass PAOx is believed to open a completely new era for these materials that receive significant interest for biomedical applications, smart materials and molecular electrical devices.

Experimental Section

Details of the synthesis, purification and polymerisation reactions are contained in the Supporting Information. All manipulations were carried out in black vacuum[29] ($< 10^{-5}$ torr) except where noted otherwise. A supplementary video showing the manipulations for the preparation of a high molar mass PEtOx is also provided as supporting information.

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Conflict of interest

R.H. and B.D.M. are listed as inventors on patent WO2016008817A1 that is based on parts of this work. R.H. is one of the founders of Avroxa BVBA that commercializes poly(2-oxazoline)s as Ultroxa®. The other authors have no conflicts to declare.

Keywords: cationic polymerisation · polymers · ring-opening polymerisation · tautomerisation

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