Unraveling the Spontaneous Zwitterionic Copolymerization Mechanism of Cyclic Imino Ethers and Acrylic Acid

Jan Steinkoenig,[a,b] Patrick A. J. M. de Jongh,[c] David M. Haddleton,[c,d] Anja S. Goldmann,[a,b] Christopher Barner-Kowollik,*[a,b] and Kristian Kempe*[d,c]*

[a]School of Chemistry, Physics and Mechanical Engineering, Queensland University of Technology (QUT), 2 George St, Brisbane, Australia. Email: christopher.barnerkowollik@qut.edu.au

[b]Macromolecular Architectures, Institut für Technische Chemie und Polymerchemie, Karlsruhe Institute of Technology (KIT), Engesserstr. 18, 76128 Karlsruhe, Germany and Institut für Biologische Grenzflächen, Karlsruhe Institute of Technology (KIT), Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany. Email: anja.goldmann@kit.edu

[c]Department of Chemistry, University of Warwick, Coventry, CV4 7AL, United Kingdom.

[d]ARC Centre of Excellence in Convergent Bio-Nano Science and Technology, Monash Institute of Pharmaceutical Sciences, Monash University, Parkville, VIC 3052, Australia. Email: kristian.kempe@monash.edu

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ABSTRACT. We report a high-resolution electrospray ionization mass spectrometric (HR ESI MS) access route leading to in-depths insight into the spontaneous zwitterionic copolymerization mechanism between cyclic imino ethers (i.e. 2-methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx) or 2-ethyl-2-oxazine (EtOz)) with acrylic acid (AA), exploiting the characteristic species accumulating during the copolymerization as well as tandem mass spectrometry (MS/MS). We demonstrate preferences in α,ω-end group formation by screening various feed ratios of cyclic imino ethers and acrylic acid (e.g. MeOx:AA = 1:1; MeOx:AA = 2:1; MeOx:AA = 1:2). Critically, a calibration curve – based on AA-MeOx-AA dimer – was established allowing for semi-quantitative determination of the end group ratios with different feed ratios of acrylic acid. The formation of, previously suggested, alternating copolymers was confirmed by MS/MS experiments. Deviations from an ideal alternating composition were found to decrease from MeOx to EtOx to EtOz. The results of (semi-quantitative) HR ESI MS and MS/MS measurements suggest, for the first time presented in such precision, a polymerization mechanism for the spontaneous zwitterionic (alternating) copolymerization indicating optimal monomer ratios and pairings.

INTRODUCTION

The spontaneous zwitterionic copolymerization (SZWIP) between a nucleophilic (M_N) and an electrophilic (M_E) monomer serves as a facile platform for the generation of sophisticated poly(ester amide)s (PEAs) and other functional and/or degradable copolymers.[1,2] Despite its early

discovery in 1977 by Saegusa and numerous reports in the 1970s and 1980s, this polymerization technique has not received much attention until its recent re-discovery by Kempe and coworkers in 2015.[2–5] These authors reported the SZWIP of various cyclic imino ethers (CIEs), such as 2-substituted-2-oxazolines (Ox), 2-substituted-2-oxazines (Oz), and acrylic acid (AA) and used it in combination with redox-initiated reversible addition-fragmentation chain transfer (RAFT) polymerization for the synthesis of functional and tunable comb polymers. The PEAs, or more precisely *N*-acylated poly(amino ester)s (NPAEs) thus obtained, represent a highly interesting polymer class as they combine the degradability of polyesters[6] with the high level of functionality of CIE derived polymers, such as poly(2-oxazoline)s (POxs). Thus, SZWIP provides access to NPAEs with tailored properties. Along with POxs, PEAs are subject of ongoing research focusing on stimuli-responsive properties as well as self-assembly for biomedical applications.[3,7–14]

However, the SZWIP mechanism has not been fully elucidated with only few publications describing the isolation of important zwitterionic intermediates.[15,16] As reported in early studies, SZWIP leads to alternating copolymers with tunable end groups. In contrast, POx-derived polymers are very well studied, including the polymerization mechanism,[17] obtained by infrared (IR) spectroscopy, nuclear resonance spectroscopy (NMR), size exclusion chromatography (SEC),[18–21] and mass spectrometry (MS) involving electrospray ionization (ESI)[22,23] as well as matrix-assisted laser desorption ionization (MALDI).[23,24] Although POxs and NPAEs feature structural similarities, it is surprising that SZWIP-prepared polymers have not yet been submitted to a precision mass spectrometric analysis allowing for a mechanism to be formulated from a post-mortem analysis of the detected species.

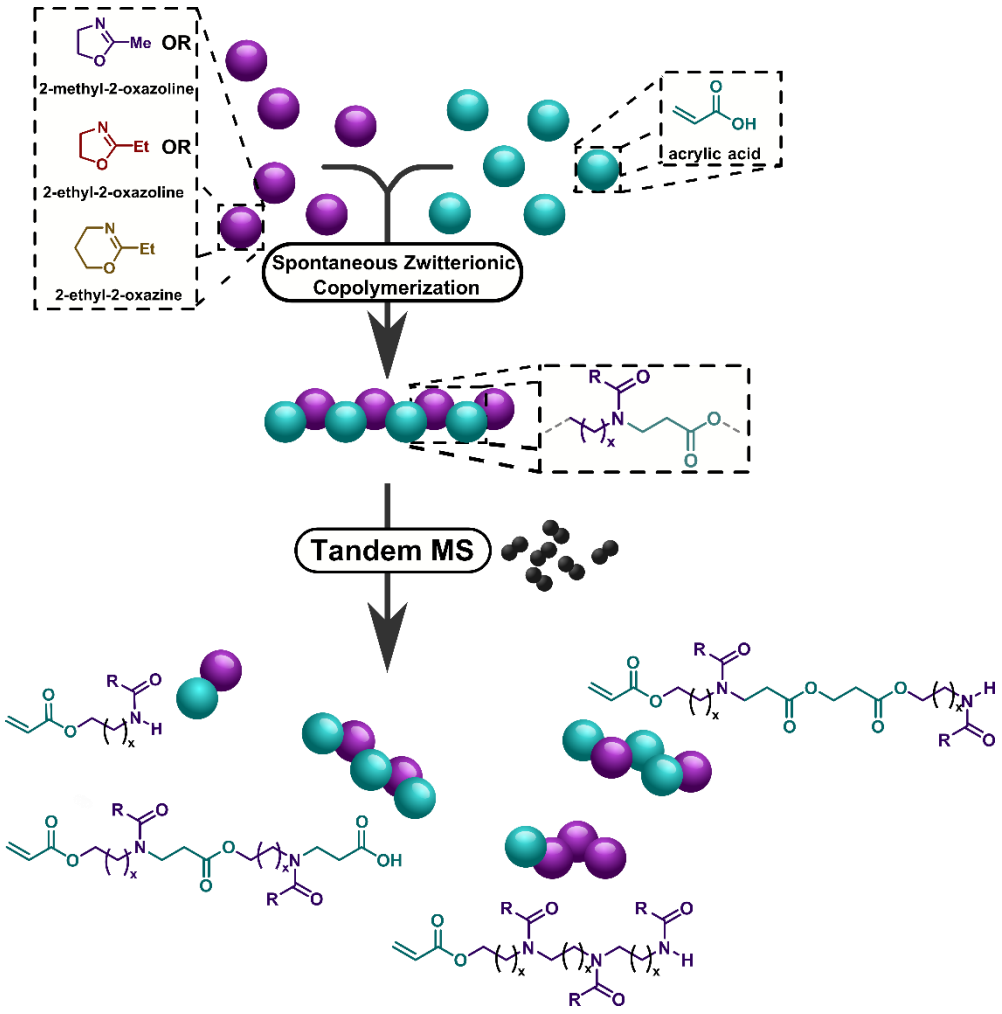
MS has demonstrated to be a very powerful technique for precision analytics in polymer science.[25] For instance, the soft ionization afforded by ESI[26] or MALDI[27] usually keeps covalent bonds

unbroken while covering a broad molecular range. Coupled to a high-resolution mass analyzer such as Orbitrap (high resolutions (up to 280000) and high ion sensitivity), macromolecules with architecturally complex structures[28] as well as polyelectrolytes[29] are accessible, and very importantly, mechanistic (polymerization) details can be revealed.[30] Especially, the collision induced dissociation (CID)[31] – with optional higher-energy collision dissociation (HCD)[32] – during tandem MS (MS/MS) measurements enables the detection of fragment ions, which can be used to deduce structural information of the precursor ion. For instance, the CID fragmentation pathway of POx has been studied intensively.[24] Since the full isotopic pattern of a single species can be stored in the HCD chamber, MS/MS measurements can give quantitative compositions of isobaric structures.

However, quantitative assessments obtained via mass spectrometry have to be evaluated with caution as the ionization efficiency may depend on chain termini (i.e. basic end groups ionize more readily), chain length (including chain conformation), salt concentration, hydrophobicity and surface activity of the analyte.[33,34] Further factors influencing the ionization are the mass-dependent ion transmission and detection (the latter can be neglected using Orbitrap). Nonetheless, two popular approaches are commonly used for ESI MS polymer chain termini quantification: (i) determination of the molar fraction based on the maximum peak height of two different species within a single spectrum;[35,36] (ii) establishment of a calibration curve based on small molecule analogues giving direct access to absolute quantities.[33,34,37] However, each of these approaches have their advantages and disadvantages. For instance, determination of the molar fraction is a work-around avoiding time consuming calibration curves (which often includes sophisticated small molecule organic synthesis). However, the molar fraction determination is governed by certain assumptions such as chain termini independent ionization and efficient ion transmission

(limiting the evaluation to single experiments).[35] If the experiment set-up is constant (i.e. producing exact same molecular weights), it is legitimate to use the molar fraction determination without calibration curve.[38] However, due to the ion transmission being mass-dependent and the fact that different polymerizations lead to different molar mass distributions, the synthesis of a reference small organic molecule and its derived calibration curve is essential to determine molar fractions for multiple experiment quantification.[34]

This present work closes a critical analytical gap for SZWIP-prepared polymers, in particular NPAEs, regarding the integrity of purely alternating structures due to its unique polymerization mechanism. **Scheme 1** collates the work that will be discussed: 2-methyl-2-oxazoline (MeOx), 2-ethyl-2-oxazoline (EtOx) and 2-ethyl-2-oxazine (EtOz) are subjected to a SZWIP procedure with acrylic acid using different feed ratios (cyclic imino ether:acrylic acid = 1:1; 1:2 (for MeOx:AA also 2:1)). The oligomers were analyzed by NMR, SEC as well as precisely by HR ESI MS. The microstructure of the SZWIP-based polymers was evidenced by HCD (with ambient nitrogen gas) at around 25 eV collision energy. The fragment ions can be unambiguously attributed to specific microstructures including consecutive incorporation of the same monomer instead of a strongly alternating incorporation. Thus, the post-mortem analysis of the specific fragment ions allows to reveal the polymerization mechanism of the SZWIP. Moreover, an important key aspect revealed by the ESI MS characterization is the end group quantification that can be tailored externally by varying the feed ratio of the monomers or even using different cyclic imino ethers.



Scheme 1. Schematic representation of the SZWIP procedure of 2-methyl-oxazoline (MeOx), 2-ethyl-oxazoline (EtOx) or 2-ethyl-2-oxazine (EtOz) with acrylic acid (AA) and their analysis via ESI MS and structural identification using tandem MS (x = 1: 2-oxazoline; x = 2: 2-oxazine).

RESULTS AND DISCUSSION

A library of oligomeric NPAEs, namely oligo(MeOx-*alt*-AA)$_n$A, oligo(EtOx-*alt*-AA)$_n$A and oligo(EtOz-*alt*-AA) has been prepared using previously reported procedures.[2,4,5,39] Specifically, CIEs were reacted with AA in different ratios as summarized in **Table 1**. The presence of rotamers

as well as the existence of different oligomeric species and end groups impede a detailed structural assessment by ^{1}H NMR, hence, the polymer composition, i.e. ratio of the two monomers, is the only information obtained.[2] Moreover, elemental analysis of the NPAEs provide further indication of the composition of the oligomers as well as the effect of different feed ratios (Tab. S2). However, in neither case, a precise structural assignment, as accessed via ESI MS, can be accomplished. The following mass spectrometric experiments have been performed on an Orbitrap mass analyzer critically surpassing the previously published data on SZWIP-prepared polymers.[2] To this end, the oligomers were dissolved in water/acetonitrile/acetic acid (1:1:0.1 (*v*/*v*)) with a concentration of 0.500 mg·mL^{-1} (the exact mass is crucial for subsequent quantifications).

Table 1. ^{1}H NMR and SEC characterization data for the oligomeric NPAEs prepared by SZWIP of different cyclic imino ethers (CIEs) and acrylic acid (AA).

CIE	CIE : AA	DP[a] (CIE/AA)	$M_{n,NMR}$[a] [g mol^{-1}]	$M_{n,SEC}$[b] [g mol^{-1}]	$Đ$[b]
MeOx	2 : 1	2.5/2.5	465	1800	1.32
	1 : 1	2.5/2.5	465	1300	1.18
	1 : 2	3/3	543	1500	1.27
EtOx	1 : 1	2.5/2.5	500	1600	1.30
	1 : 2	2/2	414	1300	1.17
EtOz	1 : 1	3/3	627	2000	1.27
	1 : 2	2.5/2.5	535	1600	1.20

[a] Determined from ^{1}H NMR analysis from the peak areas of the vinyl groups and the methylene group of CIE and AA repeating units. [b] Determined by SEC analysis.

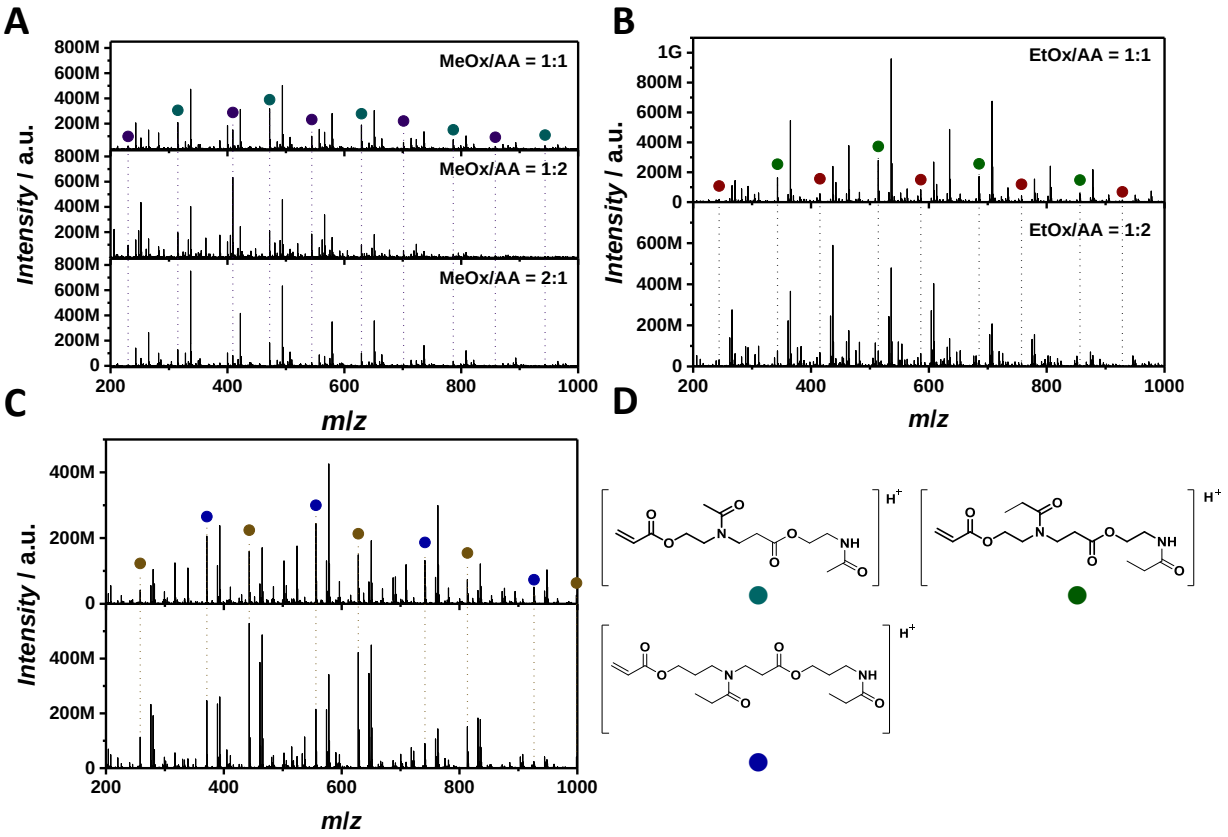


Figure 1. Overview ESI mass spectra of (A) oligo(MeOx-*alt*-AA)$_n$A with feed ratio MeOx/AA =1:1; 1:2 and 2:1; (B) oligo(EtOx-*alt*-AA)$_n$A with feed ratio EtOx/AA = 1:1 and 1:2; (C) oligo(EtOz-*alt*-AA) with feed ratio EtOz/AA = 1:1 and 1:2 recorded in water/acetonitrile/acetic acid (1:1:0.1 *v*/*v*) in a mass range between m/z 200 to 1000. The labels ●, ●, ● correspond to an acid-terminated species (H^+ ionized), and labels ●, ●, ● indicate the corresponding amide-terminated species (H^+ ionized). (D) Proposed structural assignment for the ideally alternating copolymers with α-acrylic and ω-amide end groups.

Figure 1 depicts the overview mass spectra of oligo(MeOx-*alt*-AA)$_n$A, oligo(EtOx-*alt*-AA)$_n$A and oligo(EtOz-*alt*-AA). We decided to label the proton ionized species of the ideally alternating species with respective the ω-acid chain terminus (●, ●, ●) and the ω-amide chain terminus (●, ●, ●) in order not to overload the spectra with labelling the sodiated species, which are also highly

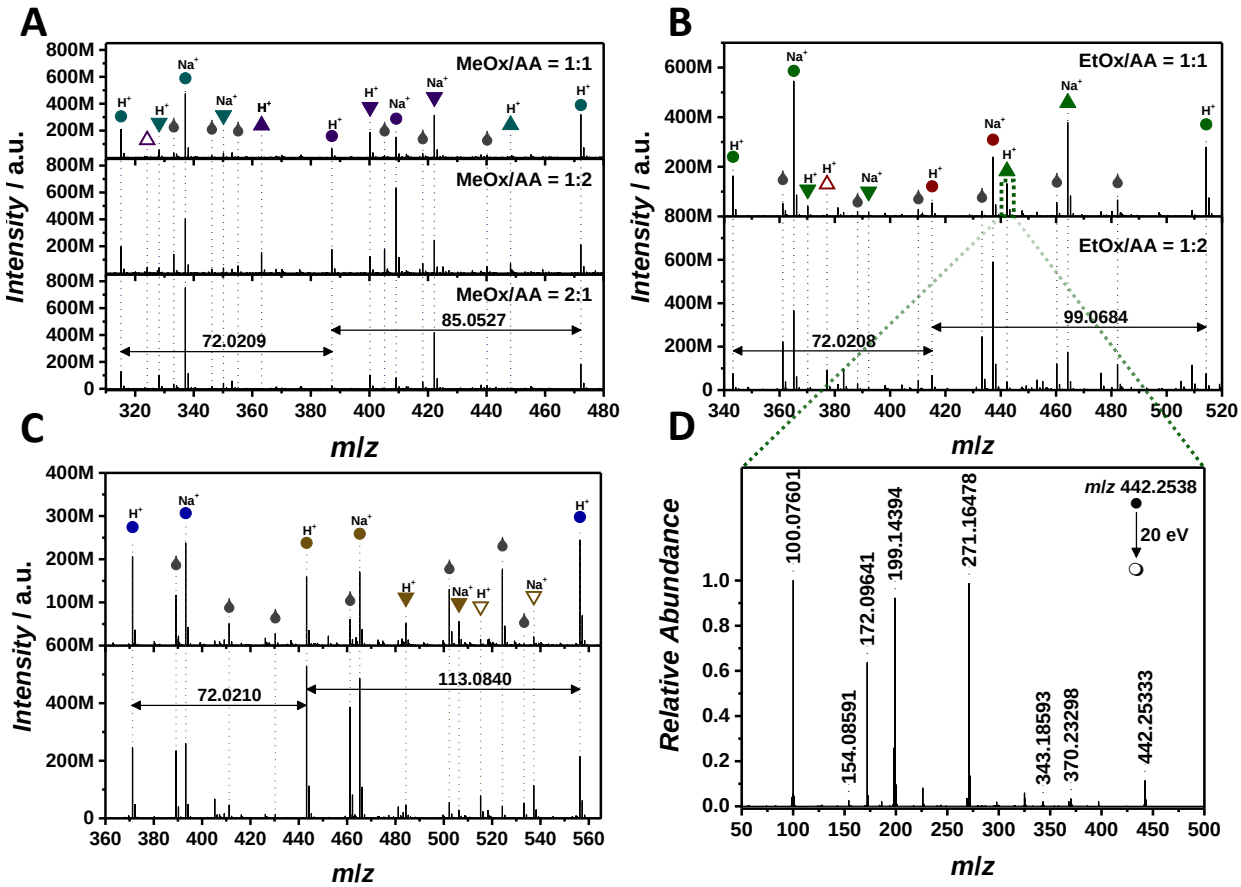


Figure 2. Expanded ESI mass spectra of (A) oligo(MeOx-*alt*-AA)$_n$A with a feed ratio MeOx/AA =1:1; 1:2 and 2:1; (B) oligo(EtOx-*alt*-AA)$_n$A with a feed ratio EtOx/AA = 1:1 and 1:2; (C) oligo(EtOz-*alt*-AA) with a feed ratio EtOz/AA = 1:1 and 1:2 recorded in water/acetonitrile/acetic acid (1:1:0.1 *v*/*v*). All relevant species are assigned in Table S3/16/25. (D) MS/MS spectrum of a oligo(EtOx-*alt*-AA)$_n$A species at *m*/*z* 442.2538 dissociated with 20 eV. The assignment (Table S21) and the fragmentation scheme (Scheme S10) clearly evidence the microstructure of the species.

abundant in the spectra (for detailed expanded spectra refer to **Figure 2**). The change in feed ratio influences the end group abundance significantly as initially observed for the MeOx/AA systems with three different feed ratios (**Figure 2A**): increasing the amount of acrylic acid leads to higher abundances of acid-terminated oligomers (e.g. represented by ●), whereas increasing the amount

of cyclic imino ethers in the feed reduces the abundance of acid-terminated oligomers drastically (quantitative assessments will be discussed later).

The expanded ESI spectra (**Figure 2A-C**) collate all species recorded in one full repeat unit (e.g. m/z(MeOx-AA)^{exp} 157.0736; m/z(MeOx-AA)^{theo} 157.0739). Apart from the discussed very abundant H^+ ionized and Na^+ ionized alternating copolymer species, further characteristic ions were detected: a low abundant peak in the MeOx/AA = 1:2 spectrum with 324.1047 m/z (labelled with △) represents oligo(MeOx$_1$-*alt*-AA$_3$). The incorporation ratio MeOx/AA=1:3 reveals that acrylic acid seems to be homocoupled to an acrylic acid dimer via a Michael addition reaction[40] before it reacts with another MeOx-AA dimer. As such a reaction preferably occurs in a surplus of acrylic acid (e.g. MeOx/AA = 1:2), the homocoupling species is of even lower abundance for the other copolymerization ratios (e.g. MeOx/AA = 1:1 and 2:1). A more important side product that has been revealed in the course of our mass spectrometric study is based on the homocoupling of cyclic imino ethers. For instance, a species at 328.1859 m/z (labelled with ▼) is almost equally abundant in each spectrum, irrespective of the copolymerization ratio and can be represented as oligo(MeOx$_3$-*alt*-AA$_1$).

In order to obtain information about the general reaction of acrylic acid as well as CIEs and the polymerization mechanism, a closer look at the microstructure is of importance. The oligomer microstructures have been revealed by employing MS/MS (**Figure 2D**), where a precursor ion was stored within the HCD chamber. Fragmentation was induced with nitrogen gas close to 20 eV. Due to many possible isobaric structures, the determination of the microstructure is a tedious process, comprising (i) the development of a feasible fragmentation mechanism;[41,42] (ii) their structural assessment; and (iii) the identification of key fragment ions unambiguously confirming the microstructure. Exemplarily performed on oligo(MeOx$_3$-*alt*-AA$_1$), such a microstructure

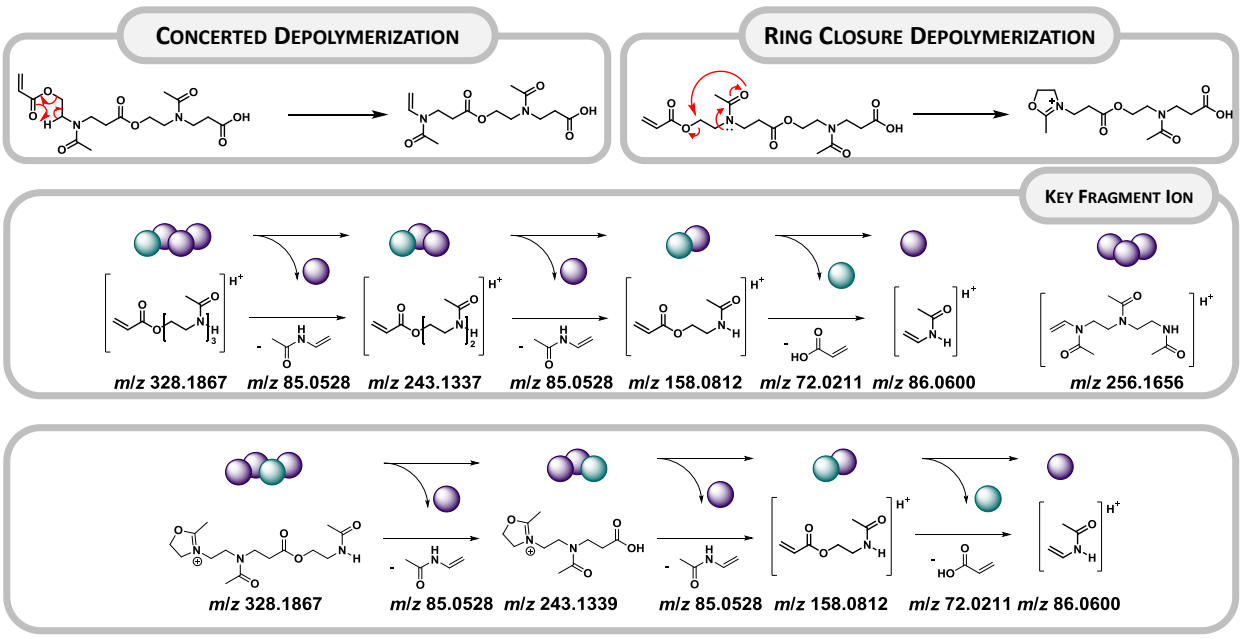


Figure 3. Proposed fragmentation (depolymerization) during MS/MS experiments revealing the microstructure of CIEs. Oligo(MeOx$_3$-*alt*-AA$_1$) having acrylic acid as chain termini (acrylic acid terminates the SZWIP polymerization) yields various fragment ions. A key fragment ion with three MeOx units connected to each other has been detected. An isobaric structure having acrylic acid incorporated into the main chain is unlikely since the cyclic iminium ether is susceptible to ring-opening reactions (e.g. water).

determination process is depicted in **Figure 3**. Two dominant fragmentation pathways are discussed in the POx-related literature:[22] (i) a concerted depolymerization via a six-membered transition state deliberating chain termini and main chain fragments alike; and (ii) a ring closure depolymerization deliberating fragments always in β-position to the acetamide structural unit. We submit that the concerted depolymerization is the favored fragmentation pathway due to specific fragmentation ions that have been identified in the course of the MS/MS study. The fragment ion 256.1656 *m*/*z* represents MeOx$_3$ confirming that the microstructure contains homocoupled MeOx. An important consequence is that acrylic acid terminates the SZWIP process by ring opening

MeOx. If the acrylic acid is incorporated into the main chain, the highly reactive cyclic oxazolinium is susceptible to ring-opening reactions with any nucleophiles (e.g. water). Yet, low abundant species have been detected (labelled with ♦ (**Figure 2**)) where the SZWIP process was terminated by ambient water instead of acrylic acid.

Close interpretation of the peaks observed in the MS spectra is of importance for the identification of isobaric structures. As discussed earlier, species e.g. labelled with ● represent amide-terminated species possessing an ideally alternating microstructure. However, tandem MS is the only technique available to precisely assess microstructural defects associated with the ideally alternating copolymer. As an example, a species identified as oligo(MeOx$_3$-*alt*-AA$_3$) prepared from a feed ratio MeOx/AA = 1:2 (m/z^{exp} 472.2295; m/z^{theo} 472.2290) is discussed. Strong signals in the MS/MS spectrum (**Figure 4**) indicate the alternating nature of oligo(MeOx$_3$-*alt*-AA$_3$). For

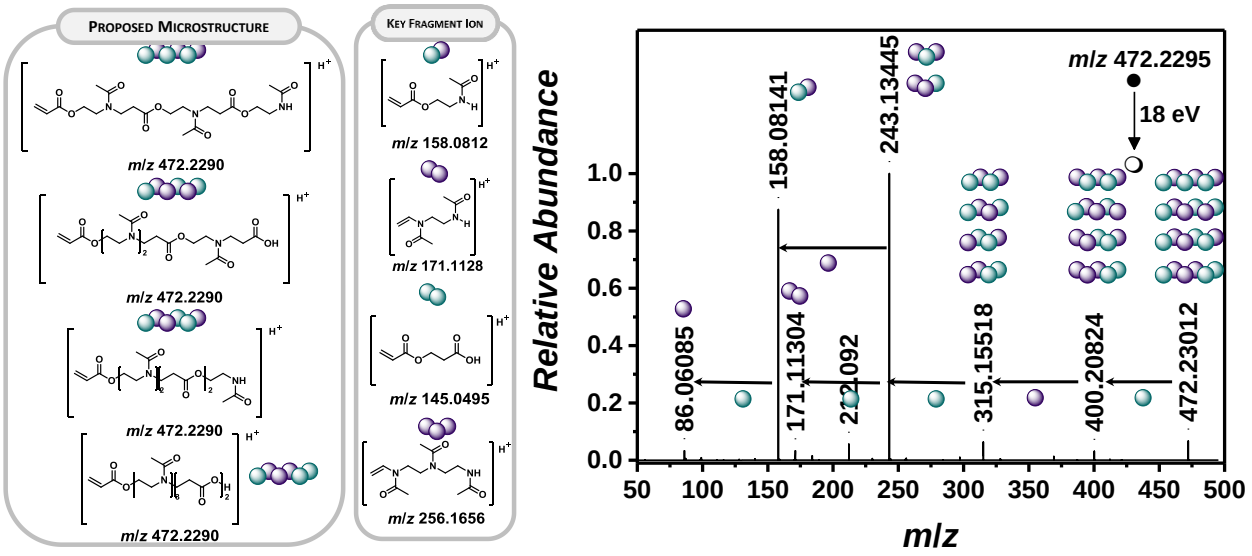


Figure 4. Proposed microstructures of oligo(MeOx$_3$-*alt*-AA$_3$) (m/z^{exp} 472.2295; m/z^{theo} 472.2290) and key fragment ions based on peaks identified. The strong alternating microstructure (●●●●●●) and a microstructure containing a MeOx-MeOx sequence (●●●●●●) can be assigned in the MS/MS spectrum.

instance, a species at 158.0814 *m*/*z* represents the AA-MeOx dimer (m/z^{theo} 158.0812; labelled with ●●). Furthermore, an alternating depolymerization sequence commencing from 472.2295 to 86.0609 *m*/*z* (MeOx monomer) is evident in the MS/MS spectrum. Strikingly, the existence of a MeOx-MeOx sequence can be evidenced by the species at 171.1130 *m*/*z* (labelled with ●●) indicating some extent of MeOx homopolymerisation during SZWIP. At 18 eV collision energy, 1.49 mol% of this species is produced as product ion.

Although MS/MS experiments are regarded to provide quantitative information based on fragment ions, spectra acquired in a full range mode (detecting several species) are of limited credibility for quantification motifs. However, it is interesting and important to evaluate the influence of different feed ratios (MeOx/AA = 1:1; 1:2; and 2:1) on the quantity of their respective end groups. As noted in the introduction, the ionization efficiency of a certain species depends on several factors,[43] which make the quantification and comparison, in particular across different samples challenging. In order to enable (semi-)quantification, a small molecule analogue, the acid-terminated dimer AA-MeOx-AA was prepared (**Figure 5A**) and used to record a calibration curve in single ion monitoring mode ranging from 0.75 mg·mL^{-1} to 0.01 mg·mL^{-1} (m/z^{exp} 230.1019; m/z^{theo} 230.1023) (**Figure 5B**).

Based on this, we were able to extract semi-quantitative information from all oligo(MeOx-*alt*-AA)$_n$A spectra with the peak used for calibration (m/z^{exp} 230.1019) being present in all feed ratios (MeOx/AA = 1:1; 1:2; and 2:1). Within the same spectrum (e.g. MeOx/AA = 1:1), we obtained semi-quantitative information based on the mole fraction determination as reported in literature (**Figure 5D**).[35] The calibration curve provides quantitative values for the acid-terminated oligomers and thus allows the semi-quantitatively determination of the mole fractions in all individual spectra (1:1; 1:2 and 2:1) and compare them with each other. Thus, the amide-

terminated species has been determined by its mole fraction always referenced to the m/z^{exp} 230.1019 species (**Figure 5A**). Semi-quantitative information from oligo(EtOz-*alt*-AA) and oligo(EtOx-*alt*-AA)$_n$A has been obtained by using the same calibration curve under the assumption that the incorporation of additional neutral CH_2 groups (either in the side chain as for EtOx or in both side and main chain as for EtOz) have limited effect on the ionization of the macromolecule. Key findings of our semi-quantification are illustrated in **Figure 5C** and further discussed in the following.

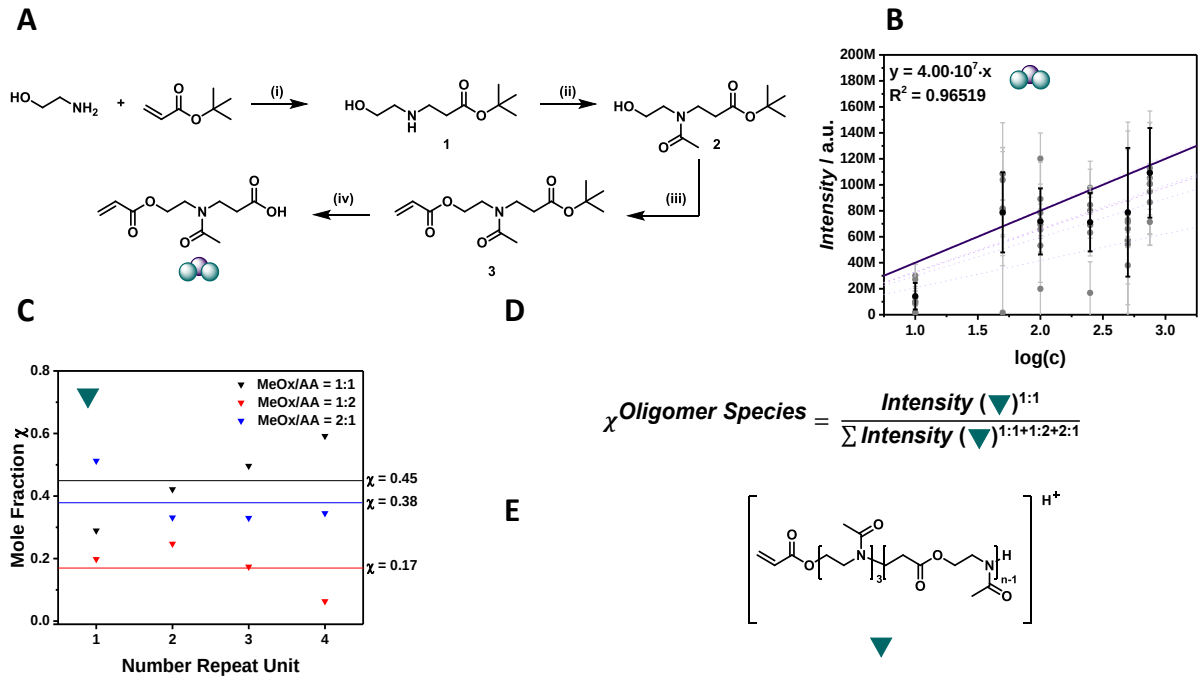


Figure 5. (A) Reaction scheme for the small molecule analogue: (i) a.t., 18 h; (ii) $(CH_3CO)_2O$, Al_2O_3, 10 min; (iii) CH_3COCl, NEt_3, DMF, 0 °C to a.t., 18 h, (iv) trifluoroacetic acid, DMF, 0 °C to a.t., 18 h; (B) calibration curve recorded in single ion monitoring (SIM) mode focusing on the H^+ ionized species (labelled with ●●●); (C) repeat unit dependent evaluation of the mole fraction; (D) equation to obtain the mole fraction (χ) and the results of one species (▼) based on the calibration; (E) general structure of species ▼.

End group quantification within MeOx: Increasing the acrylic acid content in the feed mixture increases the amount of acid-terminated oligomers (e.g. species at m/z^{exp} 387.1756, labelled with ●) from $\chi^{avg} = 0.27$ (MeOx/AA = 1:1) to $\chi^{avg} = 0.57$ (MeOx/AA = 1:2), whereas a depletion of MeOx (MeOx/AA = 2:1) yields $\chi^{avg} = 0.16$. Increasing the cyclic imino ether in the feed increases the amount of amide-terminated oligomers (e.g. species at m/z^{exp} 315.1455, labelled with ●) from $\chi^{avg} = 0.36$ (MeOx/AA = 1:1) to $\chi^{avg} = 0.49$ (MeOx/AA = 2:1), whereas MeOx/AA = 1:2 yields $\chi^{avg} = 0.20$. **Homocoupling quantification:** Species attributed to homocoupling reaction steps (e. g. the species at m/z^{exp} 328.1862, labelled with ▼) are most abundant for MeOx/AA = 1:1 ($\chi^{avg} = 0.45$), however strongly reduced for MeOx/AA = 1:2 ($\chi^{avg} = 0.17$). Generally, MeOx homopolymer sequences with up to five repeat units containing H^+ and acrylic acid end groups can be assigned in remarkable abundancies, indicating the ring-opening polymerization of cyclic imino ethers as competitive pathway. The mole fraction of these species decreases from $\chi^{avg} = 0.36$ (MeOx/AA = 1:1) to $\chi^{avg} = 0.25$ (MeOx/AA = 1:2) and increases slightly under surplus cyclic imino ether in the feed to $\chi^{avg} = 0.39$ (MeOx/AA = 2:1).

Quantification of different CIEs: Changing the 2-oxazoline from MeOx to EtOx had a compelling effect on the end groups. The amide-terminated species increase from $\chi^{avg} = 0.28$ (MeOx/AA = 1:1) to $\chi^{avg} = 0.50$ (EtOx/AA = 1:1). Strikingly, homopolymerization of EtOx decreases from $\chi^{avg} = 0.70$ (MeOx/AA = 1:1) to $\chi^{avg} = 0.27$ (EtOx/AA = 1:1). A likely explanation for the increased abundancies of amide-terminated species is that the zwitterion of AA and EtOx (represented by ●●) forms less rapidly compared to MeOx and AA (represented by ●●) (see the detailed mechanistic approach below). Thus, more ionic adducts such as $[HEtOx]^+[AA]^-$ are present in the mixture, which are responsible for the amide chain termination. Expanding the ring from a five-membered (oxazoline) to a six-membered (oxazine) ring influences the chain terminus

ratios even more drastically: amide-terminated species decrease from $\chi^{avg} = 0.28$ (MeOx/AA = 1:1) and $\chi^{avg} = 0.50$ (EtOx/AA = 1:1) to $\chi^{avg} = 0.22$ (EtOz/AA = 1:1). Further, homopolymerization of EtOz decreases from $\chi^{avg} = 0.70$ (MeOx/AA = 1:1) and $\chi^{avg} = 0.27$ (EtOx/AA = 1:1) to only $\chi^{avg} = 0.03$ (EtOz/AA = 1:1). All quantification data within each CIE (i.e. EtOx and EtOz) can be found in the SI, yet indicate the same trend as discussed previously on MeOx exemplarily.

Supported by previous literature reports and based on the post-mortem analysis of the SZWIP-prepared oligomers by mass spectrometry and tandem mass spectrometry alike, we are able to suggest a detailed mechanism for the SZWIP of CIEs and AA. In general, CIEs and AA can react in two ways: via a Michael addition reaction which leads to the required reactive zwitterionic species (**Figure 6i** and **ii**) and an acid-base reaction which results in the formation of an ion pair $[HCIE]^+[AA]^-$ (**Figure 6iii**). The acid-base reaction depends on the acidity of AA ($pK_a = 4.26$) and the basicity of the CIEs (MeOx: $pK_b = 5.77$; EtOx: $pK_b = 5.65$; EtOz: $pK_b = 6.49$).[44] As a consequence, the equilibrium of the acid-base reaction does not favor the protonation of the CIE to occur. However, as revealed by the MS analysis, the formation of an ion pair $[HCIE]^+[AA]^-$ seems to be crucial for the generation of the amide chain termini (**Figure 6vii**). Thus, the acid-base equilibrium is an important parameter to consider. As revealed by the end group determination and supported by the pK_b value, EtOx is the most probable CIE in our study to form ion pairs, represented as $[HEtOx]^+[AA]^-$ and thus form ω-amide end groups (see termination discussion below). As mentioned above, apart from reacting in an acid-base reaction, CIEs and AA can undergo an Aza-Michael addition (**Figure 6ii**) reaction followed by an intramolecular proton transfer (**Figure 6i**). The formation of the zwitterion has been experimentally confirmed in previous studies by isolation of the zwitterion.[45] Surprisingly, there is not much data available on

the Aza-Michael addition kinetics with an acrylate. Reyniers and co-workers[46] have provided calculations that estimate give equilibrium constants and kinetics of Aza-Michael additions. An important criterion for the generation of strongly alternating copolymers is the reaction rate of the Aza-Michael addition between a secondary amine and acryl derivative. Such reactions are fast (~ 10^{-3} L·mol^{-1}·s^{-1}), thermodynamically, however, the reaction is not favored (K~10^{-3} L·mol^{-1}). The driving force for the formation of the dimer is the irreversible proton transfer (K~10^{17}).[46]

After the (spontaneous) initiation under the formation of either a zwitterion or an ion pair, three propagation pathways are possible: (A) dimer-dimer reaction generating ideally alternating copolymer structures; (B) homocoupling of unreacted CIE with the dimer/oligomer producing microstructurally defect alternating copolymers; and (C) initiation of homopolymerization by the ion pair to obtain poly(cyclic imino ether)s. Pathways (B) and (C) depend on the homocoupling kinetics, with MeOx polymerizing faster than EtOx.[47] Due to the six-membered ring of EtOz, the ring-opening reaction is approximately four times slower.[48] The kinetics are mirrored by the MS study: homocoupling decreases from $\chi^{avg} = 0.70$ (MeOx/AA = 1:1) and $\chi^{avg} = 0.27$ (EtOx/AA = 1:1) to only $\chi^{avg} = 0.03$ (EtOz/AA = 1:1). Therefore, microstructural defects arising from pathway (B) are less pronounced for EtOz.

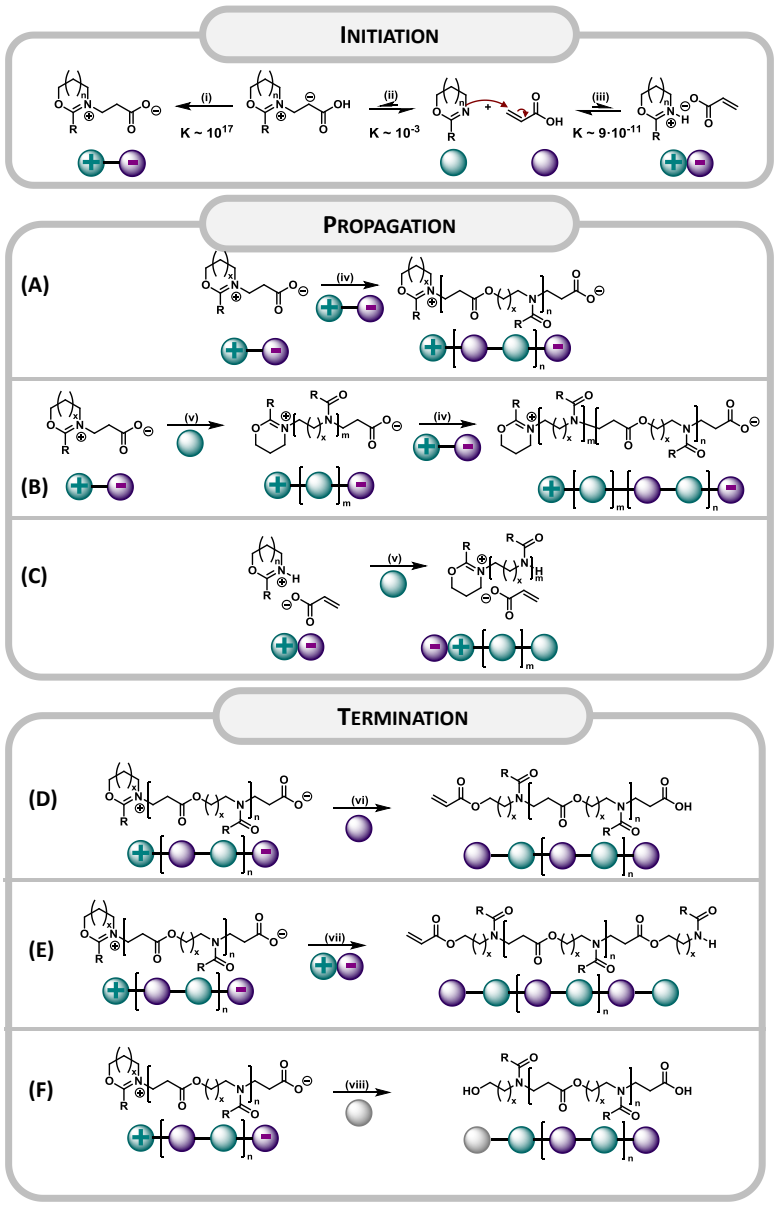


Figure 6. Proposed SZWIP mechanism based on post-mortem species identified during ESI MS and MS/MS experiments. Michael addition (MA) reaction (i, ii) to produce the zwitterionic dimer or acid/base reaction (iii) as competing reactions during initation. Alternating step-growth polymerization of MA dimers (iv), homocoupling to a MA dimer (v) or homopolymerization via consecutive homocouplings as competing reactions during propagation. Acid-induced ring opening reaction (vi) producing α-acrylic, ω-acid chain termini, two ring opening reactions (vii) forming α-acrylic, ω-amide chain termini, and water induced ring opening reaction (viii) as competing reactions during termination.

Ultimately, three termination pathways have been revealed by the mass spectrometric analysis. The majority of all zwitterionic species will be terminated by acrylic acid, which acts as nucleophile in the ring-opening reaction of the activated cyclic iminium ether and also protonates the ω-carboxylate end group (D). Thus, α-acrylic, ω-carboxylic copolymers are generated (**Figure 6vi**). As demonstrated by different feed ratios, the acid end group responses sensitively on any change in feed ratios. In contrast, the amide end group is only little influenced by changes in feed ratio rationalized by the fact that only activated cyclic iminium ethers (e.g. $[HCIE]^+[AA]^-$) will yield amide chain termini (**Figure 6vii**) (E). Thus, the formation of α-acrylic, ω-amide copolymers is observed. A third possible termination is the quenching of the active end groups by other ambient nucleophiles, such as water, which can ring-open the cyclic iminium ether (F).

CONCLUSION

We introduce an in-depth mass spectrometric investigation of oligomers produced via a spontaneous zwitterionic copolymerization of three structurally different cyclic imino ethers (CIEs) and acrylic acid (AA) yielding oligo(MeOx-*alt*-AA)$_n$A, oligo(EtOx-*alt*-AA)$_n$A and oligo(EtOz-*alt*-AA)$_n$A. Full MS spectra as well as MS/MS profiling of important species in combination with a semi-quantification obtained by a synthetically prepared AA-MeOx-AA motif unambiguously identify their microstructure with particular focus on the alternating character and end groups of the oligomers. Key findings are: (i) An excess of AA influences the amount of ω-carboxylic acid end groups significantly ($\chi^{avg} = 0.27$ (MeOx/AA = 1:1); $\chi^{avg} = 0.57$ (MeOx/AA = 1:2); $\chi^{avg} = 0.16$ (MeOx/AA = 2:1)) due to the fact that acrylic acid terminates the polymerization. (ii) An excess of CIE does not affect the amide chain terminus abundance similarly

to acid excess ($\chi^{avg} = 0.36$ (MeOx/AA = 1:1); $\chi^{avg} = 0.49$ (MeOx/AA = 2:1); $\chi^{avg} = 0.20$ (MeOx/AA = 1:2)) due to the fact that only activated CIEs will react producing ω-amide end groups. (iii) The reaction of CIEs and AA either leads to the formation of a zwitterion or an ion pair $[HCIE]^+[AA]^-$ containing an activated CIE, which strongly depends on the AA/CIE acid-base equilibrium an thus decreases from EtOx>MeOx>EtOz. Therefore, highest amide end group abundance is realized using EtOx ($\chi^{avg} = 0.50$ (EtOx/AA); $\chi^{avg} = 0.28$ (MeOx/AA); $\chi^{avg} = 0.22$ (EtOz/AA)). (iv) Importantly, homocoupling of CIEs as side reaction to the zwitterionic dimer formation introduces defects to the ideal alternating structure. The homocoupling is fast for five-membered rings (MeOx>EtOx) and four times slower for six-membered rings (EtOz) ($\chi^{avg} = 0.70$ (p(MeOx)); $\chi^{avg} = 0.27$ (p(EtOx); $\chi^{avg} = 0.03$ (p(EtOz)). Thus, oligo(EtOz-*alt*-AA)$_n$A is characterized by only a marginal amount of microstructural defects. Based on these findings, a full mechanism for the spontaneous zwitterionic alternating copolymerization of CIEs and AA has been established, which will facilitate the design of novel functional oligomers and macromonomers using the SZWIP in the future.

ASSOCIATED CONTENT

Supporting Information. Characterization methods, materials, synthetic procedures, additional mass spectra of oligo(MeOx-*alt*-AA)$_n$A, oligo(EtOx-*alt*-AA)$_n$A and oligo(EtOz-*alt*-AA)$_n$A. MS/MS spectra of various species including their fragmentation schemes. Complete data of the semi-quantification including figures for the average mole fraction determination. The following files are available free of charge.

Supporting Information (PDF)

AUTHOR INFORMATION

Corresponding Authors

*Kristian Kempe (kristian.kempe@monash.edu)

*Christopher Barner-Kowollik (christopher.barnerkowollik@qut.edu.au)

*Anja Goldmann (anja.goldmann@kit.edu)

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Unraveling the Spontaneous Zwitterionic Copolymerization Mechanism of Cyclic Imino Ethers and Acrylic Acid

Jan Steinkoenig,[a,b] Patrick A. J. M. de Jongh,[c] David M. Haddleton,[c,d] Anja S. Goldmann,[a,b] Christopher Barner-Kowollik,*[a,b] and Kristian Kempe*[d,c]*